ARCHIVES OF PATHOLOGY

VOLUME 31 MARCH 1941 NUMBER 3

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ABERRANT MUCOSA IN THE ESOPHAGUS IN INFANTS AND IN CHILDREN

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Aberrant gastric mucosa in the esophagus was first described in 1805.[1] Mucoserous glands, islands of gastric mucosa, parietal cells in such aberrant gastric mucosa—all have been independently observed in the esophageal mucosa and described in the period from 1879 to date, in American, English and German literature.[2] The material covered in the various reports has varied from 1 example to series of 30, in subjects ranging from premature infants to an adult of 68 years.

There is little written regarding the presence of ciliated epithelium within the esophagus, probably because most surveys are based on autopsies of adults or at least on series involving all ages, and, as will be brought out in this paper, such inclusions of ciliated epithelium are desquamated early in the neonatal period. The presence of ciliated epithelium in a premature infant of seven months' gestation has been noted.[3]

The incidence of gastric mucosa within the esophagus varies, according to the reports, from 0.67 to 70.0 per cent.[4] No study based on

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1. Schmidt, F. A.: De mammalium oesophago atque ventriculo, Inaug. Dissert., Halle, in off. Bätheana, 1805.

2. (*a*) Rüdinger, N.: Beiträge zur Morphologie des Gaumensegels und des Verdauungsapparates, Stuttgart, 1897, p. 27. (*b*) König: Berl. klin. Wchnschr. **31**:947, 1894. (*c*) Schaffer, J.: Sitzungsb. d. k. Akad. d. Wissensch., Math. Naturw. Cl. **106** (pt. 3):175, 1897. (*d*) Schaffer, J.: Virchows Arch. f. path. Anat. **177**:181, 1904. (*e*) Eberth, C. J.: Fortschr. d. Med. **15**:251, 1897. (*f*) d'Hardiviller: Echo méd. du Nord **1**:521, 1897. (*g*) Hildebrand, M.: München. med. Wchnschr. **45**:1057, 1898. (*h*) Kühne, F.: Virchows Arch. f. path. Anat. **158**:345, 1899. (*i*) Coffey, D. J.: Brit. M. J. **2**:840, 1900. (*j*) Hewlett, A. W.: J. Exper. Med. **5**:319, 1901. (*k*) Schridde, H.: Virchows Arch. f. path. Anat. **175**:1, 1904. (*l*) Glinski, L. K.: Bull. internat. Acad. d. sc. de Cracovie **9**:740, 1903. (*m*) Ruckert, A.: Virchows Arch. f. path. Anat. **175**:16, 1904. Schmidt.[1]

3. Healey, F. H.: J. Anat. **54**:180, 1920.

4. Taylor, A. L.: J. Path. & Bact. **30**:415, 1927. Schridde.[2k]

microscopic examinations in a large series has been published. There is little agreement as to the embryologic explanation of such mucosa, and little attention has been given to the presence of ciliated epithelium. These facts coupled with the rather frequent finding of such aberrant types of epithelium in esophagi in the departments of pathology of the Infants' Hospital, Children's Hospital and Harvard Medical School prompted a statistical survey of the subject.

EMBRYOLOGIC CONSIDERATIONS

The work of Arey [5] indicates that in the human embryo of less than 3 to 4 mm. the endodermal layer forms a hollow tube through the celomic cavities from the primitive cephala to the cauda. Down to the duodenum this tube does not close, as in lower vertebrates, but remains patent through its entire development. The primitive stomach, as yet undifferentiated, lies at approximately the level of the midneck. The respiratory groove has not yet appeared (fig. 1). In the embryo of about 3 or 4 mm. there appears on the lateral walls of the gut, in the cricoid region, a groove which is delineated caudally by a ventral groove connecting the two lateral depressions. Immediately below this point there is a slight swelling of the tube. The lateral grooves are marked internally by ridges, as is the ventral groove. The grooves and ridges mark the beginning of the separation of the lungs and trachea from the enteric canal; the swelling denotes the presence of the primitive stomach for the first time.

As the respiratory ridges enlarge and the grooves deepen, they progress in a cephalad direction, so that the lung bud separates first, to be followed by the differentiation of the tracheal tube, the whole progressing up to the pharynx. At the same time the fusiform swelling is forced caudally approximately 10 segments, rotating simultaneously, as is well known, by the growth of the heart, lungs and neck organs. The entire process is completed by the seventh week or 17 mm. stage.

It is probable that in the descent of the stomach a few rests of cells that are destined to become gastric mucosa remain in the esophageal portion of the gut and become islets of typical gastric mucosa. Whether they contain parietal cells probably depends primarily on the particular portion of the primordial fusiform gastric swelling from which they originated. The level of the esophagus at which they are found depends on their final level of descent in the course of the elongation of the esophagus concomitant with the descent of the stomach. An analogous anomaly occurs in the descent of the thyroid gland, which may leave behind cysts at various levels of the old thyroglossal duct.

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5. Arey, L. B.: Developmental Anatomy, Philadelphia, W. B. Saunders Company, 1936.

When the trachea is separated from the esophagus, both are lined by a ciliated type of epithelium. In the latter instance this eventually desquamates, usually quite completely by the time of birth.

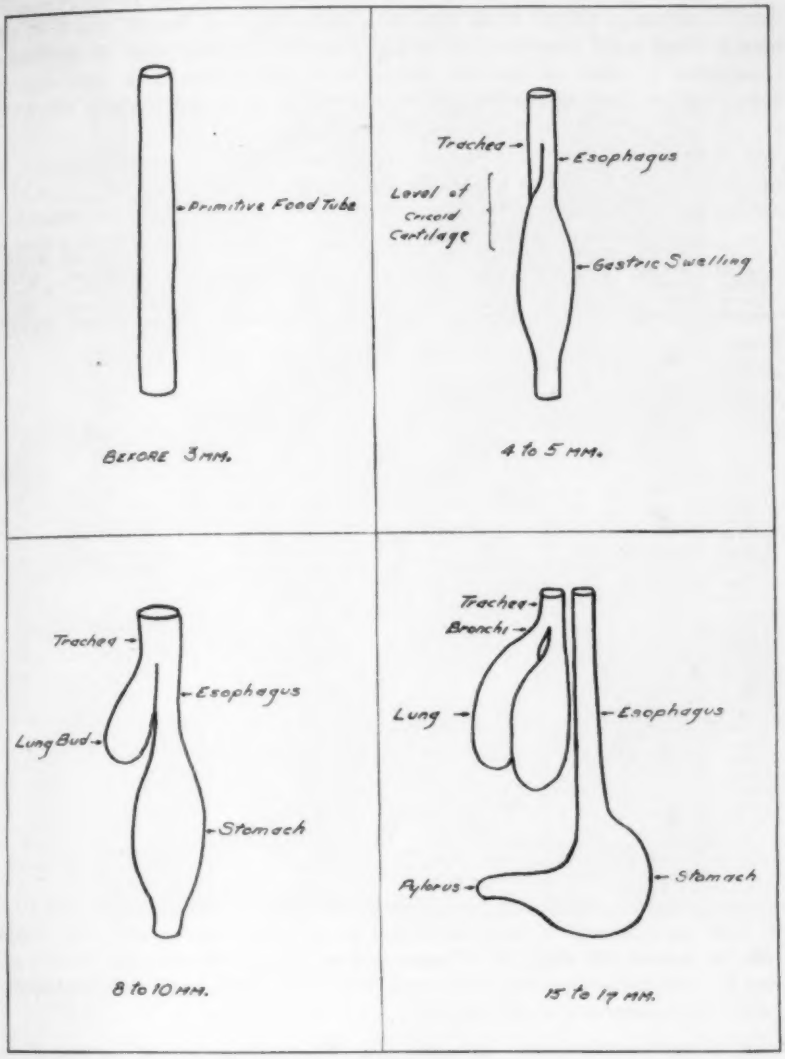

Fig. 1.—Sketches of developmental stages of the separation of the esophagus and respiratory system at crucial embryonic periods.

MATERIAL AND METHODS

The data herein reported are based on microscopic examination of sections taken from various levels of the esophagus in 1,000 consecutive autopsies of infants and children. In this series there were 547 boys and 453 girls, ranging from

prematurity to 14 years of age (table 1). A total of 1,248 sections were examined independently by us. The sections were roughly classified as to the level of the esophagus from which they were taken, on the basis of the muscle content of the wall. If the walls were composed entirely of voluntary muscle, the sections were regarded as superior third; those with both smooth and voluntary muscle were classified as middle third, and those containing only smooth muscle in the muscle coats were considered as being from the inferior third of the organ. Cognizance is taken of the fact that such a classification is at best only an approximation, since anatomists differ considerably as to the levels at which the

TABLE 1.—*Types, Incidence and Age Distribution of Aberrant Mucosa in the Esophagus*

Age at Which Death Occurred	Cases in the Series	Cases of Gastric Mucosa Without Parietal Cells	Cases of Gastric Mucosa With Parietal Cells	Cases of Superficial Ciliated Epithelium	Age at Which Death Occurred	Cases in the Series	Cases of Gastric Mucosa Without Parietal Cells	Cases of Gastric Mucosa With Parietal Cells	Cases of Superficial Ciliated Epithelium
1 day	53	8	..	38	7 mo.	35	1	1	..
2 days	21	1	..	3	8 mo.	26	2	1	..
3 days	13	1	..	1	9 mo.	32	1	..	..
4 days	15	..	..	..	10 mo.	23	..	2	..
5 days	6	..	..	..	11 mo.	19	..	..	..
6 days	7	..	..	..	12 mo.	19	1	2	..
7 days	12	2	1	..	13 mo.	14	1	..	..
8 days	6	1	..	..	14 mo.	19	..	..	..
9 days	4	1	..	..	15 mo.	14	..	2	..
10 days	5	..	..	..	16 mo.	12	..	..	..
11 days	3	1	..	..	17 mo.	20	1	..	..
12 days	4	1	..	..	18 mo.	12	..	..	..
13 days	1	..	..	..	19 mo.	12	1	1	..
14 days	12	2	..	..	20 mo.	12	1	..	..
15 days	3	..	..	..	21 mo.	6	..	..	..
16 days	1	..	..	..	22 mo.	4	2	1	..
17 days	2	..	..	..	23 mo.	4	..	..	..
18 days	3	..	..	..	24 mo.	16	1	1	..
19 days	3	1	..	..	3 yr.	38	3	2	..
20 days	1	..	..	..	4 yr.	25	..	1	..
21 days	28	5	2	..	5 yr.	25	..	..	..
23 days	1	1	..	..	6 yr.	23	..	..	..
24 days	3	1	..	..	7 yr.	20	..	..	..
25 days	1	..	..	..	8 yr.	17	..	..	..
30 days	35	3	1	..	9 yr.	17	..	..	..
2 mo.	70	1	1	..	10 yr.	11	2	1	..
3 mo.	67	3	..	..	11 yr.	10	..	..	..
4 mo.	49	3	3	..	12 yr.	9	..	..	..
5 mo.	31	3	1	..	13 yr.	2	..	..	..
6 mo.	43	7	2	..	14 yr.	1	..	..	..
Totals						1,000	63	26	42

various muscle combinations are alleged to occur.[6] With this classification, the 1,248 sections studied were distributed as follows: superior third 323, middle third 536 and inferior third 389. The sections were taken from the autopsy files and were in most instances made from tissue fixed in Zenker's acetic acid solution and stained with hematoxylin and eosin.

OBSERVATIONS

In 118 cases (11.8 per cent of the series) at least one type of aberrant mucosa was found within the esophagus. In a few cases the esophagus contained more than one type. The types, incidence and age distribution are given in table 1.

6. Arey, L. B., and Tremaine, M. J.: Anat. Rec. **56**:315, 1933.

Gastric Mucosa Without Parietal Cells.—Gastric mucosa without parietal cells was the type of aberrant epithelium most frequently found. It occurred in 63 cases, an incidence of 6.3 per cent. In 10 instances such epithelium was present as a single superficial layer (fig. 2). Examination of serial sections from the original paraffin blocks revealed continuance of the superficial type of epithelium into typical gastric glands.

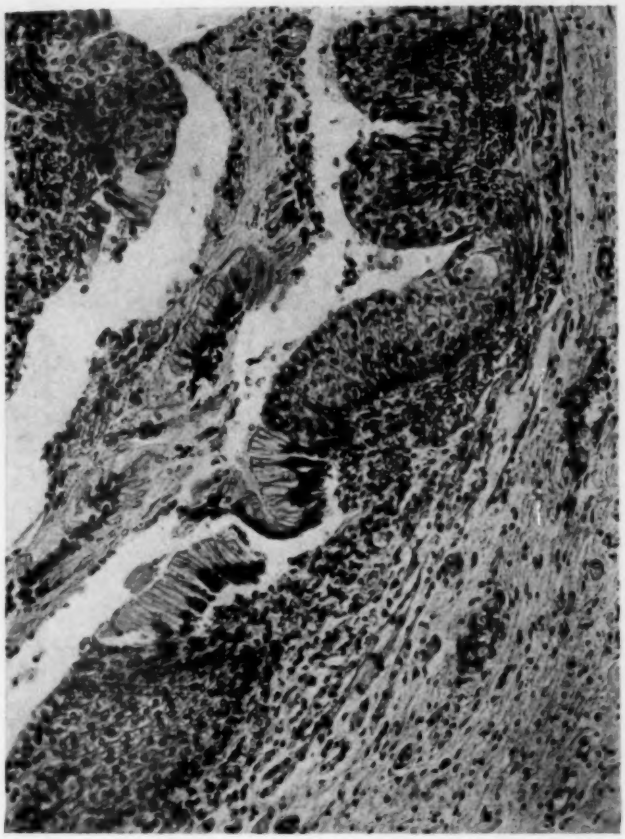

Fig. 2.—Aberrant gastric mucosa without parietal cells in the esophagus. Hematoxylin and eosin. × 233.

The preponderance of cases showing gastric mucosa without parietal cells over those with such cells may not represent the true morphologic facts, as in many such cases it was obvious that only the mouths or necks of the glands were included (fig. 3). Examination of table 2 reveals that 56 of the 63 cases involved the superior two thirds of the organ. This tends to dispel the current belief that gastric mucosa is found in the esophagus chiefly near the stomach. The sex distribution of 41 males

and 22 females is significant, for the sex distribution in the series is fairly equal, and a similar preponderance of males is evident in groups showing other types of aberrant epithelium (table 2).

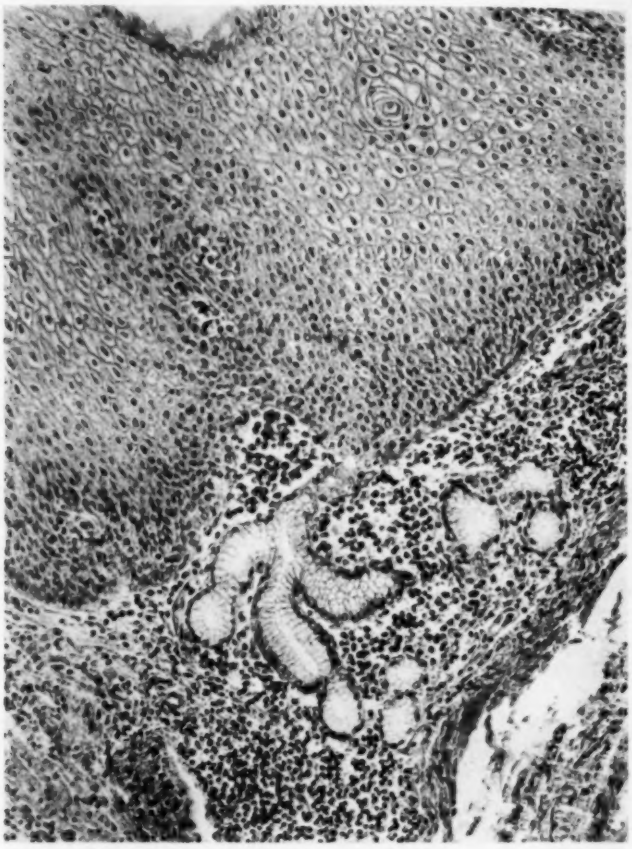

Fig. 3.—Aberrant gastric mucosa without parietal cells in the esophagus, showing only the mouth and neck of the glands included. Hematoxylin and eosin. × 233.

TABLE 2.—*Sex Incidence of Aberrant Mucosa in the Esophagus*

Type of Aberrant Mucosa	Level of Esophagus			Males	Females
	Superior	Middle	Inferior		
Gastric without parietal cells	30	26	7	41	22
Gastric with parietal cells	15	11	0	16	10
Superficial ciliated *	16	9	18	25	17
Totals	61	46	25	82	49

* In 1 case superficial ciliated epithelium was found at two levels.

Gastric Mucosa With Parietal Cells.—Gastric mucosa with parietal cells was found in the esophagus in 26 cases (fig. 4). In no instance was it situated in the inferior third of the organ. It was frequently found in association with gastric mucosa which did not contain parietal cells (fig. 4). Comparison of the age distribution of the two types in table 1 reveals a fairly parallel and scattered spread.

Fig. 4.—Aberrant gastric mucosa with parietal cells in the esophagus. Hematoxylin and eosin. × 150. Note dark-staining cells.

In no instance was gastric mucosa unequivocably identified beneath the muscularis mucosae. In several instances large islets of such epithelium extended for considerable distances into the submucosa, but invariably such structures were circumscribed inferiorly, if not completely, by at least a few fibers of the muscularis mucosae.

Superficial Ciliated Epithelium.—Figure 5 shows a typical example of the superficial ciliated type of epithelium encountered in 42 cases in

this series. In the majority of instances the subjects were premature infants (table 3). Table 1 discloses that such epithelium was not found in any child who lived more than three days; that in 38 subjects death

Fig. 5.—Superficial ciliated epithelium in the esophagus. Hematoxylin and eosin. × 1120.

TABLE 3.—*Body Length in Forty-Two Cases in Which Superficial Ciliated Epithelium Was Found in the Esophagus* *

Body Length, Cm.	Number of Cases	Body Length, Cm.	Number of Cases
30	1	41	4
31	1	42	1
34	2	43	2
35	5	44	2
36	2	45	1
37	2	48	1
38	5	49	2
39	3	50	1
40	7		

* An average length of 49 cm. has been given for full term infants (Coppoletta, J. M., and Wolbach, S. B.: Am. J. Path. 9: 55, 1933.

occurred on the first day of life, while in 3 death occurred on the second day of life. From these facts it is apparent that such epithelium is usually absent in the infant delivered at term and that when present it is rapidly desquamated.

COMMENT

In this survey some form of gastric mucosa was found in the esophagus in 7.8 per cent of 1,000 infants and children, the sections having been taken at random at various levels. Had serial sections been studied, the incidence obtained would undoubtedly have been much higher; Schridde,[2k] for example, found a 70 per cent incidence in the post-cricoid area alone. The literature contains numerous discussions of the possible clinical significance of such findings. It should be emphasized that in most instances a definite relationship has not been conclusively established.

In many of the cases in this series the aberrant gastric mucosa showed infiltration by acute and chronic inflammatory cells. In discussing the so-called peptic ulcer of the esophagus Jackson [7] stated, "The presence of islands of gastric mucosa in the lower end of the esophagus is probably a factor, but that it is an essential factor is unproved." In the same paper he mentioned the finding of such mucosa in 7 peptic ulcers in his series.

Frequently it is suggested that such aberrant gastric mucosa may play an etiologic role in carcinoma of the esophagus. In adenocarcinoma the possibility of such a relationship appears more direct; in squamous cell carcinoma the relationship is presumed to be one of constant irritation by the acid secretion. Jackson [7] stated, "Islands of gastric mucosa have been found involved in the lesion in ten of our cases of esophageal cancer." Whether these were cases of adenocarcinoma or of squamous cell carcinoma is not stated. He [8] found that carcinoma of the esophagus was nine times more frequent in males than in females, while the incidence of aberrant gastric mucosa was but one and four-tenths times more frequent in males than in females in this series.

The possible relationship of aberrant gastric mucosa to other pathologic conditions of the esophagus deserves mention. Jackson [8] suggested that esophageal scars might, in some cases, be due to repair of former inflammation in an island of aberrant gastric mucosa. It has also been suggested [9] that such areas may serve as leading points for pulsion diverticula. Gastric mucosa has been found in the relatively rare papilloma of the esophagus.[10]

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7. Jackson, C.: J. A. M. A. **92**:369, 1929.
8. Jackson, C.: Am. J. M. Sc. **169**:625, 1925.
9. König.[2b] Hewlett.[2j]
10. Brooks, H., in Tice, F.: Practice of Medicine, Hagerstown, Md., W. F. Prior Company, Inc., 1921.

Fatal hematemesis from esophageal ulcers has occurred.[11] It is conceivable that the site of such ulceration could be an island of gastric mucosa. It has been suggested [9] that esophageal cysts are caused by cystic dilatation of aberrant gastric glands, and Ireland [12] described a case in which gastric mucosa was found in an esophageal web.

Little consideration has been given to the possibility that inflammation of islets of gastric mucosa, with or without ulceration, might be a cause of unexplained substernal pain, although inflammatory reaction is frequently observed in gastric mucosa of the esophagus. Polland and Bloomfield,[13] working with human subjects, concluded that the inflation of balloons within the esophagus caused referred pain at any point from the third cervical to the eighth dorsal spinal segment, regardless of the level of innervation within the esophagus. In some instances their subjects complained of sensations akin to "heart burn." Islands of gastric mucosa have been identified frequently on esophagoscopic examination, a procedure that should be of diagnostic aid in these cases.

The presence and early desquamation of superficial ciliated epithelium of the esophagus are of academic interest only.

SUMMARY

The literature concerning aberrant mucosa within the esophagus is reviewed and the possible significance and the embryologic explanation of its presence are given.

Of 1,000 infants and children, 118 were found at autopsy to have some type of aberrant mucosa in the esophagus; 78 had either gastric mucosa with parietal cells or gastric mucosa without parietal cells or both. There were 42 in whom superficial ciliated epithelium was demonstrated; most of these were premature infants, all of whom died on or before the third day of life.

Fifty-one per cent of the aberrant gastric mucosa was found in the upper third of the esophagus; 41 per cent in the middle third, and 8 per cent in the lower third.

In 38 per cent of the cases in which the esophagus showed superficial ciliated epithelium, this was found in the upper third; in 21 per cent, in the middle third, and in 41 per cent, in the lower third.

Inflammation, with and without ulceration, was frequently present in the aberrant gastric mucosa.

No gastric mucosa was identified below the muscularis mucosae.

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11. Massive Hematemesis and Anemia in a Woman of Fifty-Five, Cabot Case 17192, New England J. Med. **204**:1000, 1931.

12. Ireland, P. E.: Laryngoscope **43**:351, 1933.

13. Polland, W. S., and Bloomfield, A. L.: J. Clin. Investigation **10**:435, 1931.

ORGANISMAL DIFFERENTIALS

FURTHER INVESTIGATIONS OF THEIR EFFECTS ON THE DISTRIBUTION OF LEUKOCYTES IN THE CIRCULATING BLOOD

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In a previous report I [1] showed that following the transplantation of various tissues there occur changes in the absolute and relative numbers of the various types of leukocytes circulating in the peripheral blood which parallel closely the reactions of leukocytes around the transplants demonstrated by Loeb [2] in extensive series of investigations. After homoiotransplantation the general response in the blood was characterized by an absolute and a relative increase in lymphocytes; after syngenesiotransplantation the response was similar but less intense and reached its maximum after a longer time. The response to heterotransplantation was of a different kind, being characterized by an early relative and an absolute increase in polymorphonuclear leukocytes; but later there occurred here also an increase in lymphocytes. This parallelism between the general blood reactions and the local reactions around the transplants suggested that the former were due also to the action of the organismal differentials which diffused into the blood and gradually reached and changed the leukocyte-forming organs.

Studies were also carried out on the effects of temperature and various chemical substances on the individuality and species differentials which are contained in the transplants, the leukocytic response being used as an indicator. It was shown that the organismal differentials were destroyed by heating at temperatures of 54 to 56 C.; freezing did not destroy the differentials. Of the chemicals tested, it was found that reagents which are known to cause changes in the constitution of proteins are especially active in injuring the organismal differentials; however, these experiments were of a preliminary nature.

From the Laboratory of Research Pathology, Oscar Johnson Institute, Washington University School of Medicine.

These investigations were carried out with the aid of grants from the International Cancer Research Foundation and the Josiah Macy Jr. Foundation.

1. Blumenthal, H. T.: Arch. Path. **27**:510, 1939.

2. Loeb, L.: J. M. Research **6**:28, 1902; **8**:44, 1902; **37**:353, 1918; **39**:189, 1918; **41**:305, 1920; Physiol. Rev. **10**:4, 1930; Biol. Bull. **40**:143, 1921; **68**:440, 1935; Science **86**:1, 1937.

Finally, it was noted in experiments in which successive transplantations were carried out that the reaction following the second transplantation was more rapid but less intense than that which occurred after the primary grafting. It was assumed that the acceleration of the reaction following the second grafting of tissues was probably due to a development of immune substances.

This work has been extended in the experiments which I now report.

EFFECT OF HOMOIOTRANSPLANTATION AND OF HETEROTRANSPLANTATION OF TISSUES ON THE WHITE CELLS IN THE CIRCULATING BLOOD OF MICE

The transplantations formerly carried out were in guinea pigs, rats, pigeons and chickens. To this group we now add experiments in mice.

TABLE 1.—*Homoiotransplantation Within Various Strains of Mice and Between Different Strains*

Donor and Recipient Strains	Experiments	Average Percental Increase in Lymphocytes	Range of Variation in Increase in Lymphocytes	Average Day of Maximum Increase
AKA to AKA	10	11.0	6-14	7.5 (4-15)*
New Buffalo to New Buffalo	6	13.0	5-18	5.2 (2-10)
Old Buffalo to Old Buffalo	6	14.3	12-16	6.0 (5-7)
CBA to CBA	6	14.3	12-17	9.0 (4-13)
A to A	10	10.0	7-12	13.6 (6-19)
AKA to New Buffalo	6	15.8	8-22	6.0 (5-7)
CBA to Old Buffalo	6	18.0	11-27	10.8 (4-13)
Old Buffalo to CBA	6	19.3	15-24	8.8 (4-11)

* The figures in parentheses represent the range of variation.

Tissues were transplanted within various inbred strains, as well as from one strain to another, in order to determine whether variations in the character or the intensity of the reaction to the homoiotransplantation corresponded to differences in the relationship between the donor and the recipient. The results obtained are shown in table 1.

From these results it may be concluded that homoiotransplantations within the same strain call forth a weaker reaction than homoiotransplantations between different strains; the average maximum lymphocytic increase in the thirty-eight experiments of the former type was 12.1 per cent, as compared with 17.7 per cent in the eighteen experiments of the latter variety. However, there does not appear to be any significant difference in the length of time necessary to obtain the highest lymphocytic count. On the average it occurred at nine and two-tenths days after transplantation when this operation was carried out within the same strain, and at eight and six-tenths days when the transfer of tissue was from one strain to another. It should be noted,

however, that when a transplantation was made between mice of the closely inbred strain A the reaction was slightly weaker than that after transplantation in other strains, and the maximum response was reached on the average thirteen and six-tenths days after transplantation; this reaction was very similar to that formerly observed following syngenesiotransplantation in guinea pigs.

Heterotransplantation was carried out from rats to 12 strain A mice. The reaction was of the kind previously described, namely, an increase in polymorphonuclear leukocytes setting in within two to four days after the transplantation and followed by a secondary rise in lymphocytes which reached a maximum between the fourteenth and the eighteenth day after transplantation. The average increase in polymorphonuclear leukocytes was 18.7 per cent, with a range of variation between 10 and 29 per cent, while the average secondary lymphocyte increase was 14.2 per cent, with a range of variation between 10 and 19 per cent.

From these experiments it may be concluded that following homoiotransplantation or heterotransplantation of tissues into mice there is a leukocytic response similar to that previously observed in guinea pigs, rats, pigeons and chickens. In mice, as in the other species, the intensity of the lymphocyte increase following homoiotransplantation depends on the relationship between the donor and the recipient. Homoiotransplantation within the closely inbred A strain induces a reaction similar to that previously observed after syngenesiotransplantation in guinea pigs. The response was more intense when transplantations were carried out between two different strains than when they were performed within the same strain.

EFFECTS OF HOMOIOTRANSPLANTATION AND HETEROTRANSPLANTATION OF EMBRYONIC TISSUES

I had previously noted that tissues of a mouse embryo which had developed to a length of approximately 15 mm. did not elicit a heterogenous type of response when transplanted to either the rat or the guinea pig; instead, there was a lymphocytic response similar to that observed after homoiotransplantation of normal adult tissues. These results suggested that the organismal differentials may not yet be fully developed in embryonal cells. In order to determine at what period of embryonal life the organismal differentials originate, tissues of mouse and rat embryos of various stages were heterotransplanted and homoiotransplanted as shown in table 2.

These experiments have been divided into two groups: The first comprises transplantations of younger embryos, which had been

removed from the uterus at the conclusion of the first third of pregnancy and the lengths of which are designated in the table. The second group comprises transplantations of tissues removed from embryos which were about three or four days removed from the end of pregnancy. In the first group the reaction was the same whether heterotransplantation or homoiotransplantation was carried out; it consisted in an increase in lymphocytes similar to that observed after homoiotransplantation of adult tissues. The older embryos behave like adult tissues, and the reaction after homoiotransplantation and heterotransplantation differed in the way which I have described. It appears, therefore, that in the younger embryos the organismal differentials have not yet fully developed and that, in particular, these embryos fail to elicit the reac-

TABLE 2.—*Effects of Transplantation of Embryonic Tissues*

Type of Transplantation	Approximate Size of Embryo, Mm.	Experiments	Cells Which Increase	Percental Increase	Average Day of Increase
Rat embryo to guinea pig.....	20	7	Lymphocytes	15.8	5.2
Mouse embryo to guinea pig....	15	4	Lymphocytes	23.7	6.3
Rat embryo to rat.....	20	4	Lymphocytes	18.0	4.3
Rat embryo to guinea pig.....	35	4	Polymorphonuclears	27.0	3.0
Mouse embryo to guinea pig....	25	4	Polymorphonuclears	19.0	4.3
Mouse embryo to rat.....	25	4	Polymorphonuclears	14.8	3.0
Rat embryo to rat.....	35	4	Lymphocytes	13.3	8.5

tion to heterogenous differentials. The latter develop in older embryos probably a short time before birth. Previous to this period heterogenous tissues elicit homoiogenous-like reactions.

EFFECT OF TRANSPLANTATION OF UNTREATED, OF HEATED AND OF CHEMICALLY ALTERED PLASMA CLOTS

In the experiments previously reported it could be shown that plasma clots, presumably free of cells, were able to elicit the typical responses to homoiotransplantation and heterotransplantation. In the experiments shown in table 3 the behaviors of plasma clots and of normal tissues are compared further.

The following conclusions may be drawn from these and previously reported experiments: Autotransplantation of plasma clots does not cause a significant increase, while homoiotransplantation and heterotransplantation induce the typical changes in the number of leukocytes in

the blood. Washing rabbit plasma clot in 0.85 per cent sodium chloride solution for three hours does not noticeably weaken the typical heterogenous reaction that follows transplantation into rats, but prolonging the washing for twelve hours weakens the reactions, suggesting that a part of the active substance has diffused into the surrounding fluid. Immersion of the rabbit plasma clot in 0.2 per cent sodium citrate or sodium fluoride does not alter the strength of the heterogenous reaction in the majority of the animals, but in some the reaction becomes negative. This can be explained by the assumption that the active substance is extracted from certain parts of the clot, perhaps from the peripheral

TABLE 3.—*Experiments with Transplantation of Plasma Clots*

Type of Transplantation	Experiments	Maximum Percental Increase in Leukocytes	Average Day of Increase
Autotransplantation:			
In guinea pigs	6	5.3 (lymphocytes)	6.0
Heterotransplantation:			
Rabbit plasma clot washed in isotonic sodium chloride solution for 3 hours and transplanted to rats	11	12.7 (polymorphonuclears)	11.1
Same, but washed in sodium chloride solution for 12 hours	6	4.0 (polymorphonuclears)	6.5
Rabbit plasma clot immersed in 0.2% sodium citrate solution for 1 hour and transplanted to rats	6	(4) 12.3 (polymorphonuclears) (2) No increase	7.7
Same in 0.2% sodium fluoride solution for 1 hour	6	(5) 15.3 (polymorphonuclears) (1) No increase	9.0
Rabbit plasma clot heated at 52 C. for ½ hour and then transplanted to rat	4	14.3 (polymorphonuclears)	8.0
Same, but heated at 54 C. for ½ hour	4	(2) 20.0 (polymorphonuclears) (2) No increase	5.0

parts, but not from others. A variable effect of heat is suggested likewise by the result of heating rabbit plasma clot at 54 C. for one-half hour; this destroyed the effect in 2 of 4 cases. Heating at 52 C. for half an hour did not diminish the effect of the clot. The temperature of 54 C. is presumably near the critical point.

In addition to the experiments in table 3, 4 experiments were carried out which showed that homoiotransplantation of plasma clot followed twelve days later by homoiotransplantation of a piece of an organ, such as the thyroid or the liver, results in an accelerated but weaker reaction to the second transplant. This is similar to the observations reported formerly, when such double transplantations were carried out with normal tissues. These experiments confirm, then, the conclusion that plasma clot, and presumably the fibrin it contains, possesses the organismal differentials just as do the normal tissues of the body.

EFFECT OF VARIOUS CHEMICAL SUBSTANCES ON THE ORGANISMAL DIFFERENTIALS

In former preliminary experiments it was tentatively concluded that the large majority of chemical substances to which the tissues were exposed previous to transplantation, and especially those which tended to denature proteins, proved injurious to the organismal differentials. These investigations have now been extended as shown in table 4.

TABLE 4.—*Effect of Chemical Substances on the Organismal Differentials*

Treatment Preceding Transplantation	Type of Transplantation	Experiments	Positive Reactions	Negative Reactions
0.9% sodium chloride in thymol for				
24 hours	Pigeon to pigeon	6	4	2
Distilled water and thymol				
(1) 24 hours	Pigeon to pigeon	4	1	3
(2) 72 hours	Pigeon to pigeon	4	0	4
Acetone for				
(1) 1 hour	Rat to rat	5	1	4
(2) 12 hours	Rat to rat	4	1	3
(3) 24 hours	Rat to rat	4	0	0
95% alcohol for				
(1) 1 hour	Rat to rat	5	0	5
(2) 12 hours	Rat to rat	4	0	4
(3) 12 hours	Guinea pig to guinea pig	3	0	3
(4) 24 hours	Pigeon to pigeon	4	0	4
A 37% solution of formaldehyde				
(1) 1 hour	Rat to rat	5	0	5
(2) 12 hours	Guinea pig to guinea pig	3	0	3
(3) 12 hours	Rat ta rat	4	0	4
Glycerin for				
(1) 1 hour	Rat to rat	4	4	..
(2) 12 hours	Guinea pig to guinea pig	3	2	1
(3) 24 hours	Pigeon to pigeon	4	0	4
(4) 72 hours	Pigeon to pigeon	4	0	4
Half-molar sodium benzoate				
(1) 1 hour	Rat to rat	5	2	3
(2) 24 hours	Pigeon to pigeon	4	0	4
(3) 72 hours	Pigeon to pigeon	4	0	4
½ saturated ammonium sulfate				
1 hour	Rat to rat	5	0	5
59% urea				
1 hour	Rat to rat	5	2	3
Half-molar ferrous chloride				
(1) 24 hours	Pigeon to pigeon	4	0	4
(2) 72 hours	Pigeon to pigeon	4	0	4
One two-hundredth molar ferric chloride				
(1) 24 hours	Pigeon to pigeon	4	0	4
(2) 72 hours	Pigeon to pigeon	4	0	4

It can be seen from these experiments that practically all the solutions used either extracted or injured the organismal differentials in part or completely. However, there are differences between different substances as to the rate at which this is accomplished. Thus, sodium chloride solution saturated with thymol removed the organismal differentials slowly, and even after twenty-four hours' immersion 4 of 6 transplants still gave a positive reaction. Immersion of thyroid in distilled water for twenty-four hours resulted in one positive reaction

in 4 experiments. Substances such as alcohol (95 per cent), solution of formaldehyde U. S. P. and ammonium sulfate, which are known to denature protein, acted very rapidly, and immersion in these solutions for but one hour was sufficient to destroy the organismal differentials. On the other hand, immersion in glycerin caused removal of the organismal differentials at a slower rate; after immersion for one hour in this substance, all the pieces reacted positively; after immersion for twelve hours, the pieces gave positive results in 2 experiments and a negative result in 1. After immersion for twenty-four and seventy-two hours, the pieces were ineffective. Occasional positive results were observed after immersion of the pieces in acetone for one or twelve hours, in sodium benzoate for one hour or in 50 per cent urea for one hour; on the whole, tissues exposed to acetone for twenty-four and seventy-two hours and those exposed to sodium benzoate for twenty-four and seventy-two hours were ineffective. Results were uniformly negative after immersion of the pieces in ferrous or ferric chloride.

It appears, then, that the organismal differentials may remain in an active state after exposure of the tissues to sodium chloride for almost twenty-four hours, or to glycerin for approximately twelve hours, but that immersion in most of the other substances tested resulted, to a variable degree, in negative reactions on the part of the transplanted tissues.

EFFECTS PRODUCED BY VARIOUS TYPES OF DOUBLE TRANSPLANTATION

It was previously noted that when a second transplantation of tissue followed a previous transplantation of the same kind (a first homoiotransplantation followed by a second homoiotransplantation or a first heterotransplantation by a second heterotransplantation) the reaction set in sooner after the second transplantation but as a rule did not rise to as high a point as that following the first transplantation. However, in a few cases the increase following the second transplantation was a little higher than that following the original grafting. It was assumed that this acceleration was due to development of immune substances. In order to test the specificity of the influence of the first transplantation on the second one, a number of additional experiments have been carried out. These are of three varieties, as follows:

1. Homoiotransplantation followed twelve days later by heterotransplantation (4 experiments)
2. Heterotransplantation followed twelve days later by homoiotransplantation (4 experiments)
3. Transplantation of casein or edestin followed twelve days later by homoiotransplantation of tissue (4 experiments with casein and 4 with edestin)

In all of these experiments the first transplantation had no influence on the second; in each instance the reaction following the second transplantation was of the type which would have taken place if it had not been preceded by a first transplantation of a different type. It may therefore be concluded that the acceleration of the reaction to the second transplantation takes place only if the first transplantation has been of the same kind; homoiotransplantation must be followed by homoiotransplantation and heterotransplantation by heterotransplantation in order to obtain acceleration.

COMMENT

Early in his investigations on the fate of transplanted tissues Loeb established the fact that it is the relationship between the individuality and species differentials of host and transplant which determines the effect of the body fluids and cells of the host on the graft. This is manifested locally about the transplant by the reaction of lymphocytes, polymorphonuclear leukocytes, connective tissue and capillary endothelium. More recently I have noted that transplanted normal tissues call forth, in addition to the local reaction, a distant action, as evidenced by changes in the leukocytes in the peripheral circulation, the latter taking a course closely parallel to the local reaction.

In the present report this more general reaction has been used in a further study of some of the characteristics of the organismal differentials. It has been possible to show that in mice, as well as in guinea pigs, rats, pigeons and chickens, the effects of organismal differentials present in normal transplanted tissues can be demonstrated by means of this reaction. Furthermore, as Loeb found in his studies, I have noted that the intensity of the reaction in the peripheral blood is dependent on the closeness of the genetic relationship of host and transplant. Thus, when the transplantation is carried out between closely inbred strain A mice there is a less intense, although longer lasting, reaction than when the transplantation is between strains not so closely inbred or between different strains.

My previous experiments have shown that the organismal differentials can be demonstrated by means of the leukocytic reaction in many tissues of the body. The present investigations have revealed the fact that they exist also in plasma clots presumably free of cells.

Through transplantation of embryos of various ages it was shown that the organismal differentials are not yet present in young embryos but develop some time shortly before birth. This lack of organismal differentials in young embryos may aid in, or be largely responsible for, the successful transfer of tissues of various species on the chorioallantoic membrane of the chick embryo, reported by several investigators.

It has furthermore been possible to prove that the organismal differentials are destroyed in the course of one hour by strong protein denaturants, such as 95 per cent alcohol, solution of formaldehyde U. S. P. and ammonium sulfate, whereas immersion in other solutions, such as 50 per cent urea, acetone, glycerin, sodium benzoate and isotonic sodium chloride, results in a slower disappearance of the organismal differentials from the tissues; in the latter cases the diminution in the effect of the organismal differentials is presumably due largely to extraction processes.

Finally, it has been possible to show that a first transplant modifies the influence which a second one exerts on the leukocytes. This effect is specific, and it becomes manifest only if homoiotransplantation is followed by homoiotransplantation and heterotransplantation by heterotransplantation, whereas different types of transplantation are ineffective in this respect.

SUMMARY

Additional data are presented which prove that the peripheral leukocytic reaction to the organismal differentials of transplanted tissue can be demonstrated in various species and that the intensity of this reaction depends on the genetic relationship of host and donor. These organismal differentials are present in plasma clots as well as in the tissues of the body; they are not yet found in young embryos, but appear some time before birth.

Strong denaturants of protein rapidly destroy the organismal differentials, whereas other types of solutions cause them to disappear more slowly.

The influence of an initial transplant on a subsequent one is specific, as indicated by the fact that the effect of a first transplant on a second one becomes manifest only if homoiotransplantation is followed by homoiotransplantation and heterotransplantation by heterotransplantation.

A YOUNG RETICULATED ARGENTOPHILIC FORM OF THE EPITHELIOID TUBERCLE

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In the laboratories of pathology at Bellevue Hospital I have had occasion to study a disease process marked by the presence in various organs, especially the lymph nodes and spleen, of small, rounded, poorly cellular bodies of peculiar histologic appearance as revealed by hematoxylin and eosin (figs. 1, 2, 3 and 4). In tissues impregnated with silver they appear as sharply demarcated reticulated argentophilic structures, in the interstices of which are relatively scant unattached cells, whose morphologic aspect is similar to that of the epithelioid cell (figs. 5 and 6). In this paper it is shown that these formations represent an early phase in the evolution of the epithelioid tubercle.

PATHOLOGIC-ANATOMIC AND HISTOLOGIC OBSERVATIONS

The bodies in question were found accidentally in the routine histologic examination of tissues removed at necropsy from a woman 66 years of age, who died four hours after admission to the hospital from heart disease of twenty years' duration. Otherwise the patient's history was negligible, and the observations at necropsy with the exception of those on the lymph nodes and spleen were of no significance in the present connection. The spleen was enlarged and weighed 240 Gm. It was firm, and its color was bluish red. The cut surface exhibited immense numbers of whitish specks, which were dismissed with a cursory comment on the numerical prominence of the "lymphoid follicles" in a subject 66 years of age. At the hili of the lungs were six or eight lymph nodes, each enlarged to a diameter of about 2 cm. Lymph nodes in the mesentery were enlarged to about the same size and number, although one of them measured 4 cm. in diameter. In them at the time of necropsy no signs of tubercle formation were detected by the naked eye, but in tissues fixed in a 4 per cent solution of formaldehyde the cut surfaces showed irregularly whitish areas, measuring 3 or 4 mm. in diameter. No primary focus of tuberculosis was found at necropsy. There was no reason to suspect such a focus, and none was looked for

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beyond the scope of routine procedure. Microscopically, the lymph nodes were practically completely replaced, only scattered islands of lymphoid cells remaining to denote the existence of a previously intact organ. In the spleen they occurred in large numbers, but the spleen was still easily recognizable as such. Next in point of frequency they

Fig. 1.—Young, loosely constructed tubercle in the walls of a pulmonary blood vessel. Hematoxylin and eosin; paraffin section; × 250.

were found in the lungs, pancreas and heart muscle; only exceptionally were they seen in the liver and kidneys.

In hematoxylin and eosin preparations the bodies in the spleen and those in the other organs named showed certain differences. Those in the lymph nodes, lungs and elsewhere were relatively richly cellular and obviously immature. Many of those in the spleen revealed varying

degrees of connective tissue replacement and were obviously somewhat older forms, although among them immature forms were numerous. Both forms stained lightly, although preponderantly, with eosin. Their size approximated that of the average island of Langerhans. In the spleen they lay exclusively in the lymph follicles. The others expressed a predilection for interstitial tissues. The younger forms were also to be seen in fibrotic areas, in the walls of small blood vessels, particularly in the lungs, or in proximity to the smaller bronchi or projecting from the

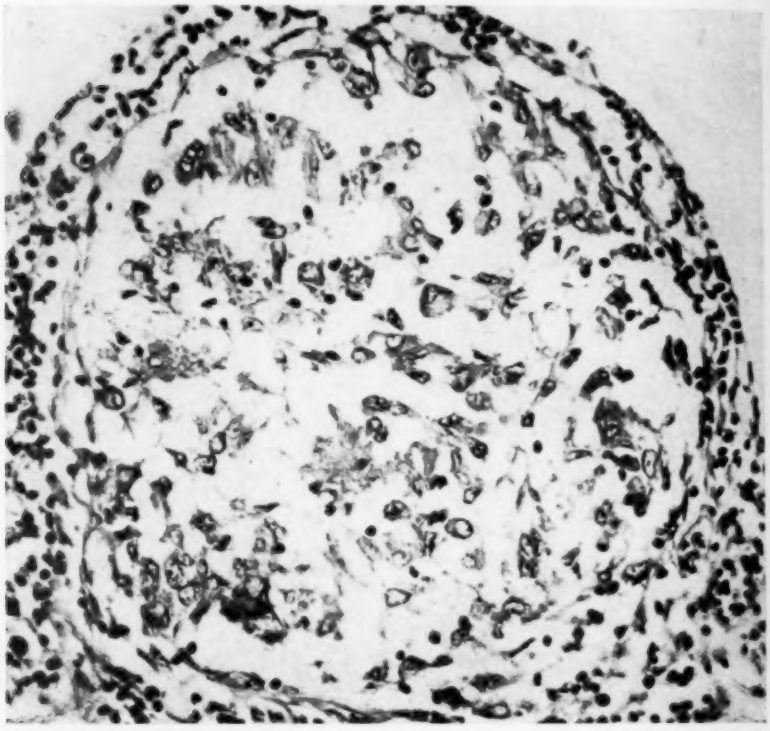

Fig. 2.—Young, loosely constructed tubercle surrounded by a thin network of poorly differentiated elongated cells, with lymphocytes in its meshes and a few scattered through the center. Note signs of early necrosis in the form of threadlike remains, amorphous debris and degenerate cells. From a lung, same necropsy as the specimen in figure 1. Hematoxylin and eosin; paraffin section; × 250.

alveolar walls (figs. 1, 2 and 4). All forms were avascular. In them, for the greater number, there was no detectable limiting structure. Others, especially in the lungs, were surrounded by a reticulum of varying thickness, composed of elongated, threadlike cells, sometimes with, but usually without, an admixture of small lymphocytes or with a mantling rich in lymphocytes. The central cells were widely separated

one from the other; they assumed fantastic shapes and were made up of faintly eosinophilic cytoplasm and rounded, lightly staining nuclei. Among them a few small lymphocytes were sometimes seen. In occasional instances multinuclear cells appeared, in which, however, the nuclei retained their indifferent staining qualities. Langhans' giant cells

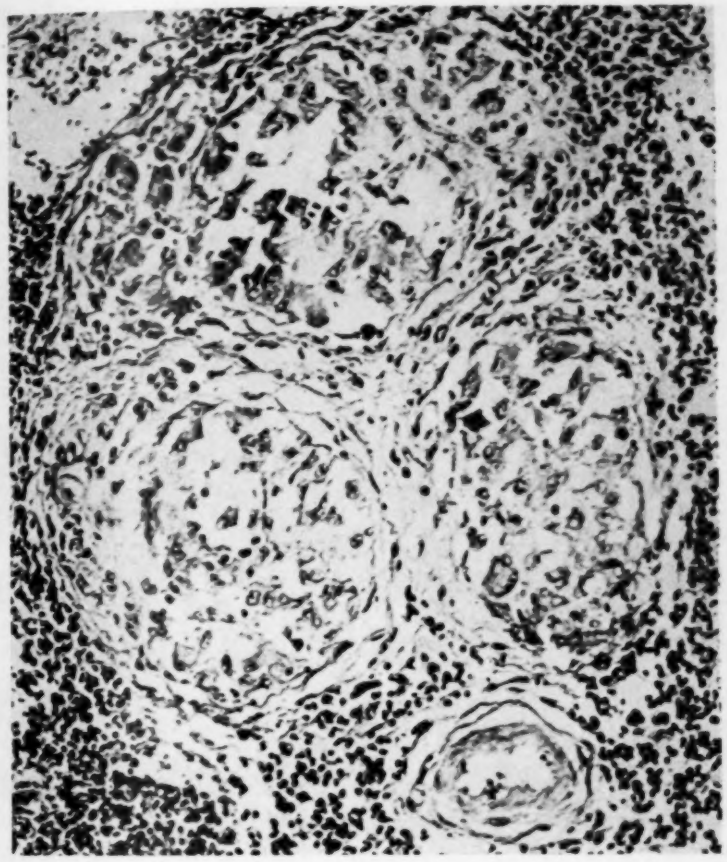

Fig. 3.—A group of young, loosely cellular, peripherally reticulated tubercles replacing a lymph follicle in the spleen. Note the central artery near the lower right corner. From the same necropsy as the specimens in figures 1 and 2. Hematoxylin and eosin; paraffin section; × 150.

were rare. There were no signs of caseation in any of the many hundreds of these bodies which were scrutinized microscopically. However, in many of them, lying among the loosely arranged cells, were pinkish-staining threadlike fragments and clumps of granular amorphous material (figs. 1, 2 and 3). At times these structureless elements were

distributed in such a manner as to suggest a broken network, and this, with the dissociation of the cells, directed attention to the loose construction of the unit as a whole. Sections impregnated with silver showed a different picture: The bodies were revealed as rounded formations bordered by argentophilic fibers of varying numbers and texture. Within the border, argentophilic fibrils were radially arranged to form small, more or less well defined compartments in which were thinly

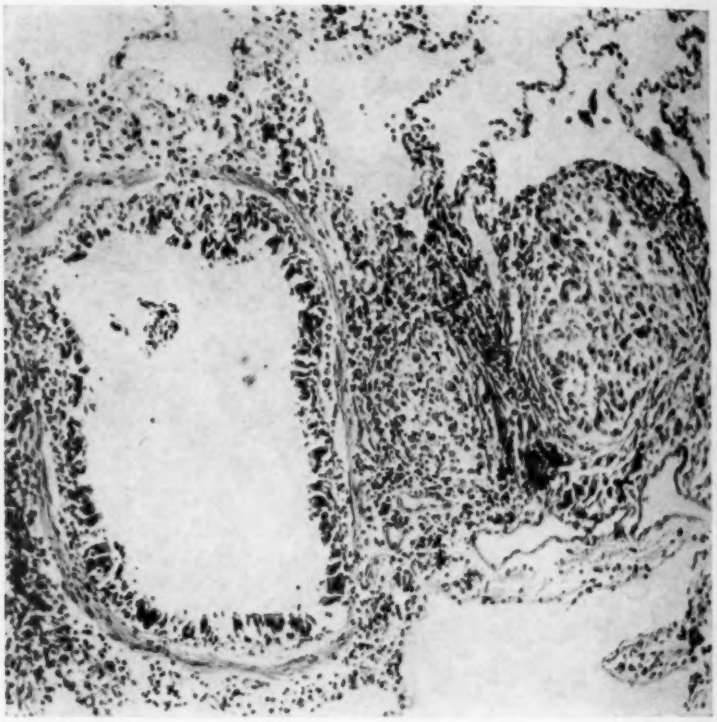

Fig. 4.—Young, loosely constructed, peripherally reticulated tubercles in proximity to a small bronchus. From the same necropsy as the specimens in figures 1 to 3. Hematoxylin and eosin; paraffin section; × 60.

scattered cells of the epithelioid type (figs. 5 and 6). In the somewhat older forms found in the spleen the argentophilic fibrils often grew profusely and became thicker, and the bodies were thus partly or almost completely replaced (fig. 7). In the argentophilic bodies, as in those stained by hematoxylin and eosin, caseation was not observed. Tubercle bacilli were not detected in any of many sections stained for them.

In a lymph node removed surgically from the neck of a man 28 years of age there were innumerable large caseous tubercles. In the walls of one of them, three immature reticulated argentophilic bodies were detected, one showing signs of early necrosis (fig. 8). Others of like caliber were scattered in some profusion through the substance of the fibrosed node, but only one of them was necrotic. Tubercle bacilli could not be found. In another instance a lymph node was removed surgically from the neck of a man 20 years of age. On cross section several

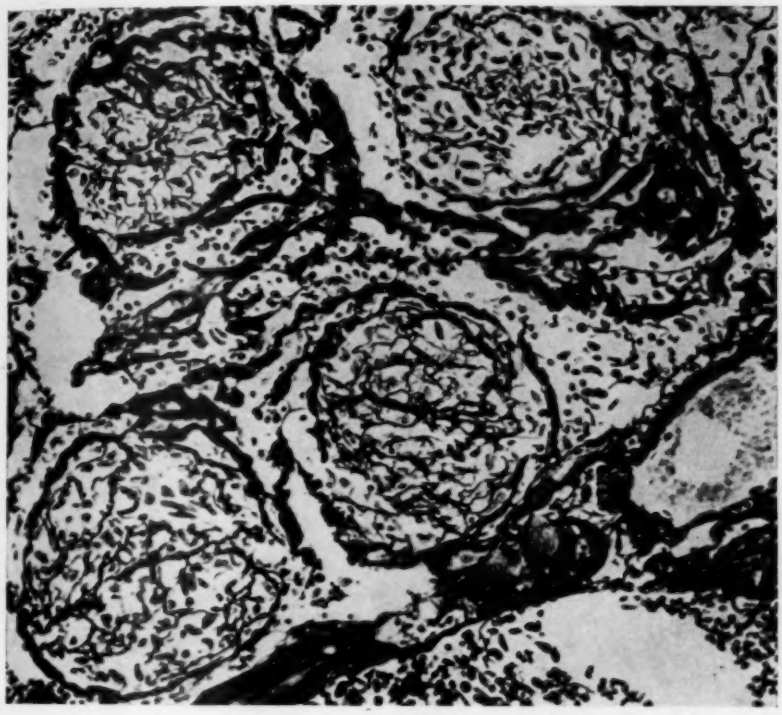

Fig. 5.—Young tubercles with borders of argentophilic fibers, central radial reticulation and unattached epithelioid cells. Early healing. From a lymph node, same necropsy as the specimens in figures 1 to 4. Silver impregnation; frozen section; × 100.

caseous foci were seen by the unaided eye. In microscopic sections impregnated with silver, literally dozens of small isolated and grouped noncaseous immature reticulated bodies were observed lying in the fibrous tissue by which the node was largely replaced. However, in the same connective tissue stroma were numbers of large caseous foci, some with and some without giant cell formation, depending on the extent of the caseous process. In the walls of one of the caseous areas

were two small, immature, noncaseous reticulated bodies and six which showed signs of early caseation. Tubercle bacilli could not be found. Numerous essentially identical examples could be cited from among the many tuberculous tissues examined in the course of this work. However, from these two observations alone the conclusion seems to me to be unavoidable that the immature, noncaseous reticulated

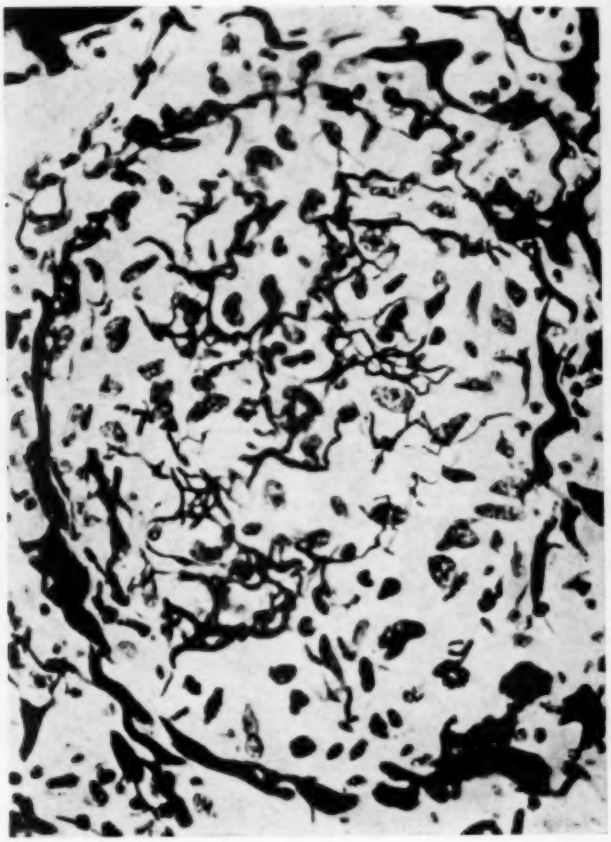

Fig. 6.—Medium-high power magnification of a young reticulated argentophilic tubercle. From the same lymph node as the specimen shown in figure 5. Silver impregnation; frozen section; × 350.

argentophilic bodies found at necropsy, and the immature, noncaseous and the caseous reticulated argentophilic bodies found in the lymph nodes under discussion, were tubercles. Nevertheless, sections of other organs known to be tuberculous were impregnated with silver. One of them, a lung, showed numerous immature reticulated argentophilic structures essentially identical with those encountered at the original

necropsy but, in addition, there were many large caseous tubercles. In the caseous areas tubercle bacilli were stained in abundance. In one of the microscopic sections of the same lung stained for tubercle bacilli three immature noncaseous reticulated bodies were found. In one of them a solitary acid-fast bacillus morphologically identical with the tubercle

Fig. 7.—A solitary tubercle almost completely healed by overgrowth of argentophilic reticulum fibers. From the spleen, same necropsy as the specimens in figures 1 to 6. Silver impregnation; frozen section; × 150.

bacillus was detected. Each of the other two showed 2 bacilli with the same structure and staining reaction. The same section revealed four additional immature reticulated bodies in process of necrosis, and all of them were rich in tubercle bacilli, single microscopic fields selected from the four showing 13, 15, 41 and 66 bacilli, respectively. That the non-

caseous reticulated argentophilic bodies found at necropsy were tuberculous is predicated on this evidence also.

Sections from the wall of a fistulous tract leading from a tuberculous empyema were stained with hematoxylin and eosin and impregnated with silver. The tissue was found to be extensively fibrosed, and

Fig. 8.—A solitary reticulated argentophilic tubercle showing signs of early necrosis. From the walls of a large caseous tubercle in a lymph node removed surgically. Silver impregnation; paraffin section; × 450.

scattered through it were large numbers of reticulated argentophilic tubercles in different stages of development and destruction. Among them were numbers of immature forms, scarcely to be distinguished from those found at necropsy. They were small and well circumscribed. The cells in them were loosely arranged and lightly stained; they assumed odd shapes, many of them resembling the classic epithelioid

variety. In addition, there were more mature, adolescent reticulated tubercles which conformed to the familiar epithelioid type except for the absence of a lymphocytic mantling. They were provided with one or more giant cells of the Langhans variety, lying in a compact matrix of epithelioid cells. On morphologic grounds alone it may be safely assumed, I think, that all of the young forms found in the fibrosed tissue from the chest wall, as well as those found at necropsy and in the various

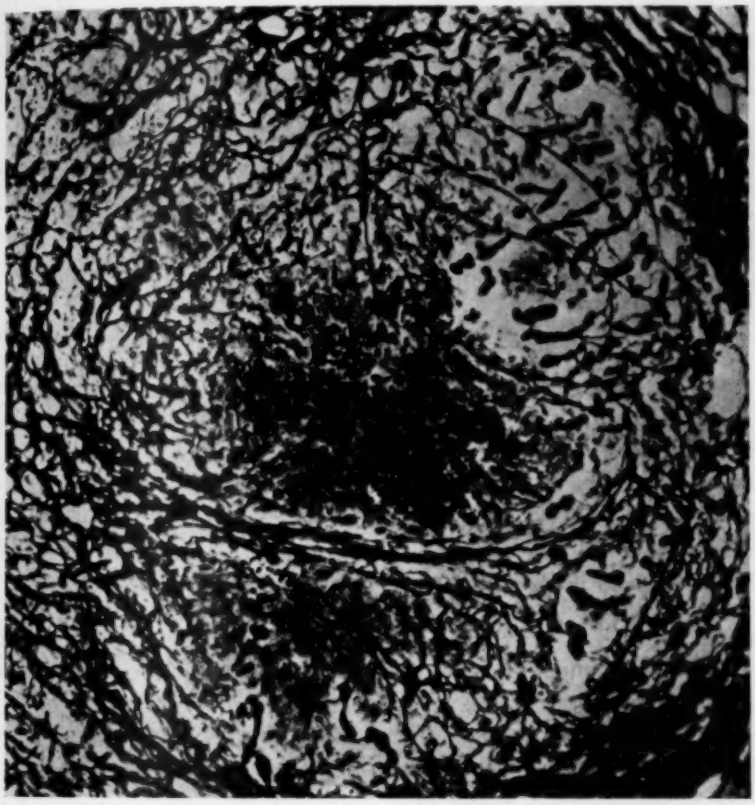

Fig. 9.—Section from a lung showing two small reticulated tubercles in the process of caseation. Tubercle bacilli were demonstrated in caseous areas in this lung and also in small, immature nonnecrotic and necrotic tubercles. Silver impregnation; frozen section; × 150.

collateral tissues examined, were tubercles and that they represented an early phase in the development of the adult epithelioid tubercle.

The behavior of the immature reticulated tubercle found at necropsy is traceable through collateral tissues by the use of simple differential stains and impregnation with silver. From the outset there is obvious

competition between the forces of repair and those of destruction (figs. 1, 2, 3, 5, 6, 7, 8 and 9). In the very young forms peripheral reticulation, when present, is revealed in sections stained with hematoxylin and eosin. Central reticulation, which is always present, is not clearly shown, if at all. Silver impregnation brings out both peripheral and central reticulation with clarity, and the disposition to heal, which is so often evinced, may be judged by the number and distribution of the reticulum fibers. In the young forms stained with hematoxylin and eosin, the cells appear as frail, indifferent creations, and many of them are obviously heading toward destruction, as shown by the presence of disintegrating nuclei, or by nuclear extrusion, and by granular degeneration of the cytoplasm. The result is manifest in the occurrence between the cells of eosinophilic stringlike bodies and granular amorphous debris (figs. 1, 2 and 3). The process represents simple necrosis as opposed to caseation. However, silver impregnation brings out what appears to be a reparative effort in the form of a fine intercellular network of argentophilic fibrils. At a somewhat later stage in the process of necrosis silver impregnation shows extensive destruction of the central argentophilic network, leaving remnants of reticulum and structureless blackish material (fig. 8).

As the young reticulated epithelioid tubercle ages and becomes larger, it assumes various familiar forms, in which the one constant feature is the presence of a delicate argentophilic reticulum. Among them is a form characterized exclusively by reticulum overgrowth, the fibers being arranged concentrically or as a complex radial meshwork or in both ways. In them the cell content is scanty. They appear to be healing forms. Another form, which is structurally comparable to the one just described, shows, however, one or more Langhans giant cells with or without small areas of caseation. Still another form shows extensive caseation. It is encircled by a reticulated wall, lying in or close to which are young tubercles of the same type as those found at necropsy and in collateral tuberculous tissues. These young tubercles may appear as well preserved structures, or they may show signs of early necrosis, and in both varieties tubercle bacilli have been demonstrated.

The frequency with which immature forms of the epithelioid tubercle were found in the lymph nodes and in the lymph follicles of the spleen at necropsy appears to be a matter worthy of note. At one necropsy, as already stated, an observer commented on the abundance of "lymphoid follicles" in the spleen of a subject 66 years of age. The remark was recalled only when microscopic examination of the spleen showed numerical and dimensional hyperplasia of the lymph follicles to an extent rarely seen so late in life. In a great many instances the follicles were histologically intact and approximated in size and number those occur-

ring in persons of an age when the follicles normally flourish. However, in a great many other instances the follicles were the seat of young epithelioid tubercles. When tubercles occur in the follicles of the spleen, there is little doubt of the derivation of the lymphoid cells which remain around or close to them. The same statement applies to tubercles replacing the germinal centers in the follicles of lymph nodes. However, the lymphocytic mantling which occasionally occurred around the young epithelioid tubercles found in the lungs removed at the original necropsy is not so easily explained. One conjecture, of course, is that the lymphocytes were attracted from some distant source. Another is that they arose in collections of small lymphocytes which are normally present in the interstitial tissues of the lung but which are so minute as not to attract attention or which, if seen, are dismissed as of no significance. Whether in human infection with the tubercle bacillus lymphoid tissue is demanded as a prerequisite for the formation of the tubercle or whether the lymphoid depot, acting in its capacity as a sedimentation basin, receives a massive dose and proliferates accordingly, is debatable. The significant fact remains that in many cases of tuberculosis involving lymph nodes in adults hyperplastic germinal areas often occur in excessive numbers.

In 1891 Trudeau isolated a strain of tubercle bacilli, which he designated RI. This strain has since been widely used in experimental work.

> When injected subcutaneously even in relatively large doses its activities are confined to the lymphoid tissues of the body. Intravenous or inhalation infection is followed by visceral lesions which attain full maturity and then undergo more or less complete resolution, leaving, however, residual disease in the lymph nodes. For many experimental purposes such an organism as the RI strain is extremely useful, but it is obvious that deductions based upon the reactions to it cannot be applied to all tubercle bacilli.[1]

In the case here presented of a woman 66 years of age, the spleen weighed 240 Gm., which is about 100 Gm. in excess of the normal weight. In it were great numbers of young epithelioid tubercles and, I should guess, more than half of them when impregnated with silver showed signs of partial or almost complete healing by overgrowth of reticulum fibers. In the enlarged peribronchial and mesenteric lymph nodes, as well as in the lungs, signs of healing, as brought out by silver impregnation, did not approach in extent those met with in the spleen similarly treated. Irrespective of the probable location of the primary focus of tubercle formation as indicated by the size of the spleen and the different

1. Baldwin, E. R.; Petroff, S. A., and Gardner, L. S.: Tuberculosis: Bacteriology, Pathology and Laboratory Diagnosis with Sections on Immunology, Epidemiology, Prophylaxis and Experimental Therapy, Philadelphia, Lea & Febiger, 1927, p. 62.

ages of the tubercles in the spleen as compared with those in the lymph nodes, it appears to me that certain of the changes in the lymphoid tissues are comparable to those produced in experimental animals by the RI strain of tubercle bacilli. By this, of course, I do not mean to imply that the reticulated argentophilic epithelioid tubercle is a reaction peculiar to infection with the RI strain.

The young reticulated argentophilic form of the epithelioid tubercle is of common occurrence. That it has not been previously described is due, I think, to the perfunctory manner in which tubercles are apt to be examined. I am free to confess that at first I did not recognize its nature. I am now convinced that I have often seen it but have never observed it. Once recognized, it is easily identified and may be found in affiliation with productive tuberculous lesions wherever they occur.

SUMMARY

There is a young form of epithelioid tubercle which is circumscribed and centrally reticulated by argentophilic fibers. In its interstices are relatively scant numbers of unattached epithelioid cells. That it is a tubercle is shown by the presence in it of acid-fast bacilli having the morphologic characteristics of the tubercle bacillus. It expresses a predilection for lymphoid tissues, especially the lymph nodes and the spleen, but may be observed in various other organs. It may occur as a small independent body lying at the edge or incorporated in the substance of the mature reticulated epithelioid tubercle or in the limiting walls of large caseous foci. It shows concomitant signs of necrosis and of healing, apparently from the moment of its inception. It may finally caseate or undergo partial or complete replacement by overgrowth of reticulum fibers.

MORPHOLOGIC ALTERATIONS IN THE REGRESSING BROWN-PEARCE TUMOR AND THEIR RELATION TO CHANGES DUE TO IRRADIATION

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AND
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In 1923 Pearce and Brown [1] demonstrated that when the Brown-Pearce carcinoma, a readily transplantable malignant tumor of the rabbit's testicle, is transplanted into the rabbit's skin, the tumor grows but soon regresses and eventually disappears, leaving the animal refractory to subsequent transplantation. This observation has been substantiated on several occasions.[2] Saphir and Appel [3] have shown further that in animals bearing testicular and cutaneous tumors, and also metastases, regression of the cutaneous tumor is followed by regression of the testicular and metastatic tumors.

During the course of the latter experiments, a number of sections were taken from the regressing tumors and submitted to careful microscopic study. On the completion of this study it was noted that the histologic changes observed in the regressing tumors of immunized animals bore a marked resemblance to the changes found in tumors following irradiation. Because of this striking similarity, it was thought advisable to report the results of this study.

MATERIAL AND METHODS

Biopsies on tissues taken from 28 rabbits with regressing and regressed Brown-Pearce testicular, cutaneous and metastatic tumors formed the basis for this study. Regression of the testicular tumors and of their metastases was effected by intracutaneous transplantation as previously described.[2]

From the Department of Pathology of the Michael Reese Hospital.

This investigation was aided by grants from the Women's Board of Michael Reese Hospital and the A. B. Kuppenheimer Fund.

1. Pearce, L., and Brown, W. H.: J. Exper. Med. **37**:811, 1923.

2. Besredka, A.; Magat, J., and Besnard, P.: Compt. rend. Acad. d. sc. **201**: 303, 1935. Besredka, A., and Gross, L.: ibid. **202**:1217, 1936. Besredka, A.; Magat, J.; Laval, P., and Besnard, P.: Ann. Inst. Pasteur **56**:125, 1936.

3. Saphir, O., and Appel, M.: Am. J. Cancer **38**:55, 1940.

When regression of the tumors began, specimens were taken for biopsy at varying intervals. The tissues thus obtained were fixed in 10 per cent solution of formaldehyde U. S. P., embedded in paraffin and stained with hematoxylin and eosin. Many of the sections were also stained for connective tissue with Van Gieson's stain and for calcium with von Kossa's stain. In several instances serial sections were cut of portions of a tumor or from the margin between the tumor and the surrounding tissues of the host. Regressing and regressed cutaneous and testicular tumors and also regressing and regressed metastases to the liver, kidney, lymph nodes and eye were examined in this way.

In order to determine the fate of tumor transplanted into a refractory animal, intratesticular transplantations were done in a series of 6 rabbits that had been immunized by intracutaneous transplantation. At varying intervals tissues were taken from these transplants and examined by the technics described.

RESULTS

Before the histologic changes in these regressing tumors are described in detail, it should be pointed out that there were no striking microscopic differences between the testicular tumors and metastases which regressed following intracutaneous implantation and regression, and these regressing tumors of the skin. Also, there were no essential cytologic differences between the aforementioned regressing tumors and the resorbing transplants in refractory animals.

The gross and microscopic characteristics of the actively growing tumor have been described in detail by Brown and Pearce.[4] The tumor was usually very cellular; i. e., it had a scant connective tissue stroma, in which were embedded many closely packed cells. The latter were not arranged in any characteristic fashion but were distributed in closely packed nests and cords. Occasionally there was a tendency toward the formation of imperfectly shaped alveolar structures. The relation of stroma to tumor cells varied considerably in different tumors and in different organs. The cells were epithelial in type and exhibited marked variation in size and shape, as well as in intensity of staining reaction. They varied from round or oval to polygonal. They had abundant coarsely granular eosinophilic cytoplasm and pale round or oval vesicular nuclei. Each of the latter had one or more prominent nucleoli, and many were found in various stages of mitotic division. Tumors growing in various organs exhibited considerable morphologic variation but in general adhered to the morphologic pattern described. At the border between the tumor and the surrounding tissues of the host there were always present a number of newly formed blood vessels, a proliferation of fibroblasts and a few lymphocytes and endothelial leukocytes. The proportions of these elements varied according to the length of time which had elapsed between transplantation and the examination of the tumor.

4. Brown, W. H., and Pearce, L.: J. Exper. Med. **37**:601, 1923.

The most striking feature of the regressing tumor was the marked degeneration of tumor cells and the necrosis. When a regressing cutaneous or testicular tumor was excised, the firm, fleshy, somewhat lobulated tissue one observes in the actively growing tumor was found to be replaced by a soft, necrotic, cheesy, friable tissue. This was relatively avascular and did not bleed as readily as did the actively growing tumor.

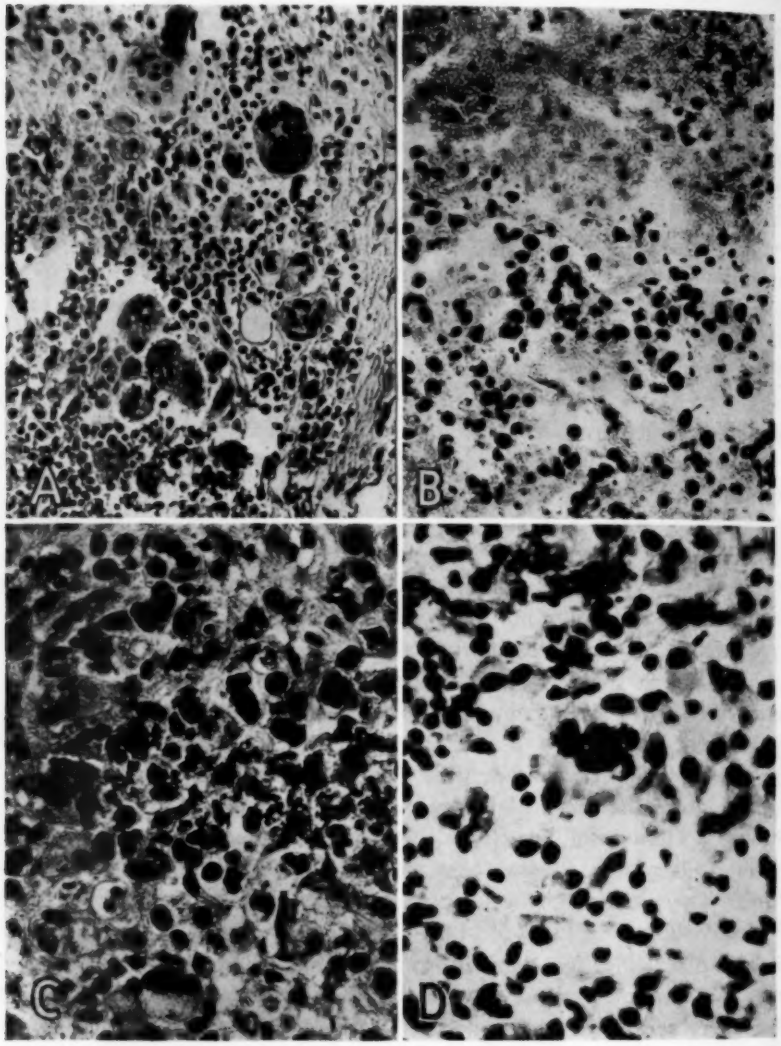
Histologically, in place of the richly cellular tumor growth found in the actively growing lesion there were large, structureless, amorphous basophilic masses, embedded in which were varying numbers of ghostlike cellular remnants. The latter were in varying stages of degeneration. In some only the cell outline containing coarsely granular or vacuolated cytoplasm, without any nuclear remnant, was visible. In others, pyknotic, poorly defined nuclei were seen. Fragmentation of the nuclei was a common finding. Intermingled with these were occasional fairly healthy tumor cells, which were similar to those already described. Mitotic figures, however, were not often present. Typical tumor giant cells were frequently seen. Degenerated tumor cells were widely scattered, singly, in pairs or occasionally in small clusters. The dense, compact masses of cells seen in the actively growing tumor were never seen in the regressing growth. The entire tumor was usually diffusely infiltrated by many lymphocytes and also by a few polymorphonuclear leukocytes and macrophages. The intensity of the inflammatory reaction varied considerably, depending on the site of the tumor, the presence or absence of ulceration and, in general, the readiness with which secondary infection could take place.

Throughout the sections of the regressing tumors large tumor cells were often present which contained either large hyperchromatic or pyknotic nuclei. Often a gradual change from such pyknotic nuclei into calcified nuclei was observed. In these fields one frequently found tumor giant cells, the nuclei of which were also prone to these calcific changes. Occasionally such calcified nuclei appeared to have fused, forming a large calcific nuclear mass surrounded by a thin rim of cytoplasm. These tumor giant cells were entirely different from those to be described subsequently.

Foci of calcification distinct from the calcified nuclei just described were also present quite constantly. They were especially prominent close to or within necrotic areas. Occasionally large calcified plaques were found. Sometimes small calcific deposits were seen within foreign body giant cells. It should be emphasized that occasionally the calcific changes were also observed in the actively growing tumors.

As already mentioned, areas of necrosis, varying in extent, were found throughout the sections. They were most commonly seen in sections taken from the central portions of tumor masses. Occasionally, small necrotic foci were observed surrounded by surviving sheets of

tumor cells arranged in a somewhat "peritheliomatous" fashion. It was interesting to note that between the more centrally placed areas of necrosis and the adjacent normal structures of the host there were,



A, foreign body giant cells with only occasional tumor cells; × 170. *B*, pyknotic tumor cells and necrosis; × 330. *C*, calcified nuclei of tumor cells; × 520. Note the calcified chromatin particles. *D*, calcified tumor giant cell; × 520. All the tissues shown are hematoxylin-eosin preparations.

almost invariably, well preserved tumor cells in small foci or even larger sheets. Areas of necrosis were not observed as a rule in the peripheral portions of the tumors.

Many giant cells of the foreign body variety were often found at the margins of the tumors. They contained large masses of cytoplasm and marginally placed, somewhat vesicular nuclei. Occasionally such cells were observed within the tumor, at a distance from its periphery. In some areas individual giant cells were found; more often giant cells were present in large masses. Often a definite attempt toward the formation of a foreign body granuloma could be made out, and here and there a true foreign body granuloma was encountered.

A diffuse cellular infiltration was usually present between the border of the tumor and the normal tissues of the host. It consisted of many endothelial leukocytes and lymphocytes and varying numbers of polymorphonuclear leukocytes and foreign body giant cells of the type just described. In this region there was also sometimes a luxuriant growth of young fibroblasts, as well as some newly formed connective tissue. A striking feature in these sections was the paucity or almost complete absence of newly formed blood vessels. Only after several adjacent sections, cut in serial fashion, were studied, was it possible to recognize an occasional small blood vessel.

The most striking histologic feature of the regressed tumor was the marked fibrosis at the site of the previous tumor growth. In all of the organs examined which had previously been the site of tumor growth, dense bands of fibrous connective tissue replaced the parenchymal structures. Embedded in this connective tissue were distorted epithelial elements, distributed singly or in small clusters. These had granular or vacuolated, faintly eosinophilic cytoplasm. Their nuclei were pyknotic or did not stain at all. These cells resembled very closely the degenerated tumor cells found in the regressing tumor. Foci of calcification and calcified nuclei were often encountered. In some instances complete disappearance of the tumor occurred with hardly any appreciable scar formation. This was seen most frequently in the liver and kidney. The tendency toward scar formation was most marked in the testicle, skin and eye.

Following complete resorption of the cutaneous tumor, only a slight thickening of the skin remained at the site of the intracutaneous transplantation. Regression of the testicular tumor often left a slightly enlarged, firm, fibrous testicle in which no tumor was grossly recognizable. At the site of a regressed metastatic tumor in eye, lymph node or kidney grayish white scar was found, in which tumor could not be grossly identified.

COMMENT

In summary, the outstanding histologic features in these regressing tumors were: degeneration and necrosis, the latter being particularly prominent in the central portions of the tumors; long survival of a marginal zone of fairly well preserved tumor cells; calcific nuclei and

tumor giant cells with calcific nuclei; foci of calcification; presence of foreign body giant cells, with attempts toward the formation of, and the actual formation of, foreign body granulomas; presence of many lymphocytes, polymorphonuclear leukocytes, endothelial leukocytes and many fibroblastic cells; rarity of newly formed blood vessels.

It may be wise to point out that in nearly every discussion of the fate of a tumor transplant in an animal refractory to the development of a tumor one continually encounters such terms as "immunity" or "tumor-immune animals." Whether the fact that the transplant does not "take" or soon regresses permits one to speak of true immunity, as is often done, or, perhaps better, of immunity in the broadest sense of the term remains questionable. It seems much more appropriate to use the term "refractory animal."

A number of the changes listed as features of regressing tumors have been noted previously, particularly by Russell,[5] as early as 1908. This author obtained refractory animals by using mice which had been inoculated unsuccessfully with tumor tissue, or normal mice treated with mouse blood or with embryo emulsion, or mice in whom tumors had grown for a time and then been resorbed. He particularly stressed that in these animals the outstanding feature is the failure of the new host to supply a vascular stroma. This leads to necrosis of the transplant with the exception of the cells about the periphery, which are able to obtain their nourishment by a process of diffusion from the host tissues.

The absence of an angiofibrous stroma appears to be a characteristic feature of regressing tumor transplants in tumor-refractory animals. Symeonidis[6] also emphasized this fact. He stressed that quick and complete encapsulation of the transplant combined with the closing of the circulation is significant. By this procedure, the tumor tissue becomes isolated, as if it were foreign material. The foreign body giant cells and foreign body granulomas which we have described provide additional evidence for the assumption that the refractory host tends to treat the transplant as a foreign body.

In the leukocytic infiltration which was found throughout the regressing tumor and also at its periphery the predominating cell was the lymphocyte, although a number of polymorphonuclear and endothelial leukocytes were also invariably present. Whether or not the lymphocytes are so characteristic that one should ascribe to them the special significance which Symeonidis did cannot be decided. Grand and Chambers,[7] on the other hand, found that the most frequently encountered cell type in the regressing transplants was the granulocyte.

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5. Russell, B. R. G.: Scient. Rep. Invest. Imp. Cancer Research Fund **3**: 341, 1908.

6. Symeonidis, A.: Virchows Arch. f. path. Anat. **304**:115 and 271, 1939.

7. Grand, C. G., and Chambers, R.: Am. J. Cancer **39**:211, 1940.

It is interesting to note that in most instances the necrotic region was observed in the central portion of the transplant and that a marginal zone of tumor tissue, close to the normal host tissue, survived. This was also stressed by Symeonidis,[6] who, because of this finding, concluded that the blood serum and the tissue fluids of the refractory animal do not possess specific carcinolytic properties.

It should be mentioned at this point that necrosis was also occasionally encountered in the Brown-Pearce testicular tumor in the normal, nonrefractory animal. It must be emphasized, however, that spontaneous regression of this tumor in our rabbits was only rarely observed with our tumor under the conditions maintained in our laboratory. However, in these instances the necrosis was not confined to the central portions of the tumors as in the refractory animals but was found throughout the tumor, in the peripheral as well as in the central portions.

As stated, in addition to degeneration and necrosis of the tumor cells, tumor giant cells with hyperchromatic or pyknotic nuclei, calcified nuclei and calcific deposits were constantly present in the regressing transplants. These changes bore a striking resemblance to those seen in tumors following irradiation. Ludford [8] stated that when a tumor is irradiated, the sensitive cells disintegrate in a characteristic manner. Degenerated and necrotic tumor cells appear. Other cells become enlarged, and giant multinucleated forms are found. In the nuclei, chromosomal alterations resulting in fragmentation and fusion into irregular masses are observed.

It is well known that necrosis occurs in tumors following irradiation. Pyknosis and karyorrhexis are quite commonly seen in these tumors. However, in addition to these changes, other degenerative alterations occur after irradiation with divided doses. These changes were recently studied and described by Melnick and Bachem.[9] These observers found that some of the tumor cells become altered in their chromosomal structure, an alteration which results in the formation of giant cells with hyperchromatic nuclei. These cells do not disintegrate by pyknosis and fragmentation, but in a specific manner, namely, by calcification of their nuclei. The authors stated also that the sensitive cells in a tumor are those which eventually show pyknosis and fragmentation of the nuclei. Such cells may form tumor giant cells, but the refractory tumor cells are those which show gradual transformation into cells with calcified nuclei. The authors stressed that the former changes occur especially in tumors treated with single massive doses of radiation, whereas the latter occur following treatment with divided doses. In our regressing tumors

8. Ludford, R. J.: Scient. Rep. Invest. Imp. Cancer Research Fund **10**:125, 1932.

9. Melnick, P. J., and Bachem, A.: Arch. Path. **23**:757, 1937.

in refractory animals definite evidence was found of changes which might occur following irradiation with single or divided doses. Both types of changes were found: Pyknosis, fragmentation of the nuclei, formation of tumor giant cells, tumor giant cells with hyperchromatic nuclei and often transition of such nuclei to calcified nuclei were all observed.

As mentioned previously, the similarity between regressing and irradiated tumors was striking. Vorlaender [10] made a histologic study of spontaneously regressing tumors in mice and also found changes similar to those observed in tumors receding after irradiation. It would appear perhaps that the changes attributed to irradiation effect are not the direct result of irradiation, but occur in those instances in which—whatever the reason may be—the tumor gradually becomes smaller until eventually it completely disappears. The presence of these calcific changes may simply be an indication of the beginning regression of the tumor.

The explanation of the mechanism of the regression of these transplanted tumors and in some cases also of their metastases is of the utmost importance and has been discussed on several occasions by various writers. Burgess [11] and also Tyzzer [12] considered the inflammatory reaction to be an important factor in the failure of transplants to grow in refractory animals. The recent work of Grand and Chambers [7] confirmed this. The latter stated that they considered the essential feature of this reaction to be the accumulation of blood granulocytes and offered the suggestion that the destruction of tumor cells is due in part at least to liberation of proteolytic enzymes by the disintegration of the granulocytes. Russell [5] attributed the regression of the transplant in the nonsusceptible animal principally to the failure of the host to supply a vascular stroma. He ascribed this failure of vascularization to one of two factors. The host tissues may be so altered by the immunization process that they do not react to the stimulus of the cancer cells, or the latter may be altered in such a way that they can no longer incite a specific reaction. The immunity was considered to be directed against the chemotactic influences exerted by the cancer cells on the connective tissue of the host, and the absence of a specific stroma reaction in refractory animals was thought to be due to the suppression of the chemotactic properties of the cancer cells. Thus the transplanted cells are deprived of their powers of assimilation and growth, and die soon after inoculation into the new host. Russell

10. Vorlaender, K.: Strahlentherapie **18**:564, 1924.

11. Burgess, A. M.: Rep. Caroline Brewer Croft Cancer Com., Harvard M. School **5**:249, 1909.

12. Tyzzer, E. E.: J. M. Research **32**:201, 1915.

concluded that in the circulating fluids or tissues of the resistant animal there is a substance which inhibits chemotaxis and allows these transplanted cells to proliferate for eight to ten days but eventually injures them so that they can no longer withstand the encroaching fibroblasts of the new host. The fibroblasts penetrate between the cells and produce scar tissue instead of the vascular connective tissue framework required by the parenchymal cells. The latter become compressed and eventually die.

As stated, Symeonidis [6] concluded from the finding of healthy tumor tissue between the centrally located necrotic portion and the surrounding normal tissues of the host that the blood serum and the tissue fluids of the refractory animal do not possess specific carcinolytic properties.

From our study we are unable to offer any new explanation for the regression of these tumors in resistant or immunized animals. However, it must be stressed that there is nothing liberated by or contained within the regressing tumor which might be considered responsible for its regression. This became clear after a number of regressing cutaneous Brown-Pearce tumors had been excised and transplanted into the testes of normal rabbits. These all grew successfully, producing general carcinomatosis of the same type as that produced by transplants taken from actively growing tumors. Wylegschanin [13] found similarly that after removal of a resorbing transplant from a resistant animal, in which it did not grow, the transplant did grow well in a susceptible animal. Grand and Chambers [7] showed that portions of such a regressing transplant when removed from the refractory host grew well in tissue culture.

SUMMARY

The gross and cytologic characteristics of resorbing Brown-Pearce carcinoma transplants in refractory animals and resorbing tumors in animals rendered resistant or "immune" by intracutaneous transplantation are described. The outstanding changes were: degeneration and necrosis in the central portions of the tumors, while the marginal zones survived for a considerable time; infiltration by many lymphocytes and by a few polymorphonuclear and endothelial leukocytes; formation of foreign body giant cells; formation of tumor giant cells, with calcification of nuclei and deposition of calcium throughout the regressing transplants; failure of the new host to supply a vascular stroma. These changes are compared with those described following irradiation of tumors with divided doses and certain striking similarities pointed out. Various theories explaining the mechanism of the production of this immunity are discussed.

13. Wylegschanin, A. I.: Ztschr. f. Krebsforsch. **38**:99, 1933.

THE MYOEPITHELIUM IN SWEAT GLAND TUMORS

DISTRIBUTION, HISTOLOGY, EMBRYOLOGY AND FUNCTION

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Until recently, relatively little attention has been paid by pathologists to the contractile epithelial cells, although these cells have been known to anatomists for a long time. These cells, called myoepithelium, are present in the mammary glands, the eccrine and apocrine sweat glands, Moll's glands of the eyelid and in the cerumen glands of the ear. Similar contractile epithelial elements, called basket cells, are found in the salivary and lacrimal glands. Their presence in the ducts of the pancreas has not been definitely ascertained.

The myoepithelium in the sweat glands was first described by Kölliker,[1] in 1889. These cells form the outer of the two layers of cells constituting the gland and are therefore situated inside the basement membrane, from which they can be separated. They are spindle-shaped cells, triangular on cross section, 30 to 90 microns long, with an elongated, rod-shaped nucleus and longitudinal cytoplasmic fibrils. Their morphologic features and staining qualities are those of smooth muscle fibers. The inner cell layer of the gland, in contrast to the myoepithelium, is formed by a single row of secreting epithelial cells, the shape of which varies from cuboid to columnar.

The longest axis of the contractile elements runs parallel or slightly tangential to that of the gland. In the apocrine sweat glands they form an almost continuous outer layer, while in the eccrine sweat glands there are spaces between the single cells. Through these spaces the adjacent epithelium reaches the basement membrane. The myoepithelium and the epithelium are connected with each other by thin intercellular bridges. In the excretory ducts of the sudoriferous glands the myoepithelium disappears and is substituted by a second layer of epithelium.

The ectodermal origin of the myoepithelium is accepted and stressed by all investigators. The development of the sweat glands begins during

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From the Department of Pathology, Free Hospital for Women.

1. Kölliker, cited by Hoepke, H.: Die Haut, in von Möllendorff, W.: Handbuch der mikroskopischen Anatomie des Menschen, Berlin, Julius Springer, 1927, vol. 3, pt. 1, p. 61.

the fourth month of intrauterine life, but no information is available in regard to the time when the myoepithelium appears first in these glands.

As to the function of the myoepithelium, it is stated that this tissue by its contraction aids in expelling the product of the gland. The contraction may also cause an apparent stratification of the overlying secreting epithelium.

REVIEW OF THE LITERATURE

According to Günther,[2] proliferation of the myoepithelium in the mammary glands has been reported by Langhans, Jüngst, Dreyfuss, Krompecher, Masson and Bertschli. Günther[2] found myoepithelial proliferation in 12 of 64 breasts taken from women who had come to autopsy for varying causes. This material was taken without correlation as to the presence of chronic cystic mastitis. Similar proliferation was noted in 13 of 53 surgical specimens showing chronic cystic mastitis.

Abramow[3] described an adenomyomatous breast in which the stroma showed smooth muscle fibers, sometimes in such number as to resemble leiomyoma. Durante and Roulland[4] reported a tumor of the female breast which they considered as having been derived from the myoepithelium, while Gaudier, Granclaude and Lambert[5] described a tumor of the female breast which was diagnosed as malignant myoepithelioma.

Hamperl[6] frequently found neoplastic proliferation of the myoepithelium, for which he coined the term "myothelium," in the mammary glands of older women and sometimes in such benign tumors of the breast as adenoma, fibroadenoma and intraductal papilloma.

Besides the carcinosarcoma and other peculiar tumors of the female breast, this author also studied the mixed mammary tumors of dogs and found similar marked proliferation of myoepithelium in all these neoplasms.

Having stressed the ectodermal origin of the myoepithelium, Hamperl[6] asserted not only that the epithelium is capable of giving rise to the myoepithelium but that in this case it may produce directly, or by previous transition through the latter, other tissue of mesenchymal character, such as reticulum and collagen fibers, mucus and cartilage ground substance. Thus he considered these tumors as having been derived from epithelium rather than from different tissue anlagen or from an embryonic rest.

The presence of myoepithelium in the tumors of sweat glands has been mentioned by only a few authors. Darier,[7] in the original description of the multiple sweat gland tumors of the skin, mentioned the presence of myoepithelium.

2. Günther, R.: Virchows Arch. f. path. Anat. **300**:449, 1937.

3. Abramow, S. S.: Zentralbl. f. allg. Path. u. path. Anat. **12**:926, 1901.

4. Durante, G., and Roulland, H., cited by Hamperl.[6]

5. Gaudier, Granclaude and Lambert, M., cited by Schultz-Brauns, O.: Die Geschwülste der Brustdrüse, in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1933, vol. 7, pt. 2, p. 303.

6. Hamperl, H.: Virchows Arch. f. path. Anat. **305**:171, 1939.

7. Darier, J.: Arch. de méd. expér. **1**:115 and 267, 1889.

Grynfeltt and Aimes[8] described 2 sweat gland tumors, the first of which was considered benign, while the second showed adenocarcinoma. In both the myoepithelium predominated as the main element in the formation of the tumor, as is well shown by their drawings. While in the first tumor the myoepithelium displayed the usual morphologic characteristics, its appearance in the second was atypical. The nuclei were round instead of rodlike, and the cytoplasm had lost its longitudinal fibrils. Pelagatti[9] mentioned only briefly the presence of myoepithelium in a tumor diagnosed adenocarcinoma of the sudoriferous glands. Loos,[10] in his paper on carcinoma of the skin appendages, mentioned the presence of myoepithelium in his own case, in which, however, these cells did not assume particular importance. He stated that they might show regressive changes or be entirely absent in rapidly growing tumors, and quoted Blavet di Briga as having made the same observation. He expressed the opinion that the participation of the myoepithelium in the formation of sweat gland tumors was not unusual because of the close histogenetic relationship between these cells and the epithelium proper, although he gave no further details.

It is probable that more cases of neoplastic myoepithelial proliferation in sweat glands have been described in the literature but have not been recognized as such.

MATERIAL

A series of 10 sweat gland tumors was studied, 6 from the Peter Bent Brigham Hospital, 1 from the Children's Hospital, and 2 seen at the Mallory Institute of Pathology, at the Boston City Hospital. Another was submitted to our laboratory from the outside. Dr. S. B. Wolbach, Dr. S. Farber and Dr. Frederick Parker Jr. allowed me to use their material, Dr. F. A. Simmons supplied the clinical history of his case and Dr. A. T. Hertig gave helpful advice.

The material was fixed in Zenker's fluid with the exception of our own, which was submitted to the laboratory fixed in 10 per cent solution of formaldehyde U. S. P. The blocks were embedded in paraffin. The following stains were used: hematoxylin-eosin, eosin-methylene blue, Mallory's aniline blue, Mallory's phosphotungstic acid–hematoxylin, Van Gieson's solution of trinitrophenol and acid fuchsin, Lawson's modification of Masson's tetrachromate, Weigert's iron-hematoxylin, Weigert's elastic tissue stain, Foot's reticulum stain.

In 3 of the 10 tumors the myoepithelium participated actively in the formation of the tumor. The study of these 3 tumors forms the basis of this report.

FIRST CASE

A white girl 10 years and 5 months old presented a bean-sized, firm, slightly tender lump within the thickness of the right cheek. This was noted first two months prior to admission. It did not increase in size and was not attached to the skin or to the oral mucosa.

Gross Observations.—The excised specimen consisted of a globular firm mass, measuring 1.7 by 1.1 by 1.1 cm. It was covered with a thin connective tissue capsule, which transmitted a pearly gray color. The cut surfaces were uniformly

8. Grynfeltt, E., and Aimes, A.: Bull. Assoc. franç. p. l'étude du cancer **11**:91, 1922.

9. Pelagatti, V.: Tumori **8**:434, 1934.

10. Loos, H. O.: Arch. f. Dermat. u. Syph. **174**:465, 1936.

firm, moistly glistening and of a whitish yellow color. Near the periphery a small cystic cavity was found, which measured 2 mm. across and contained some clear sticky mucoid fluid.

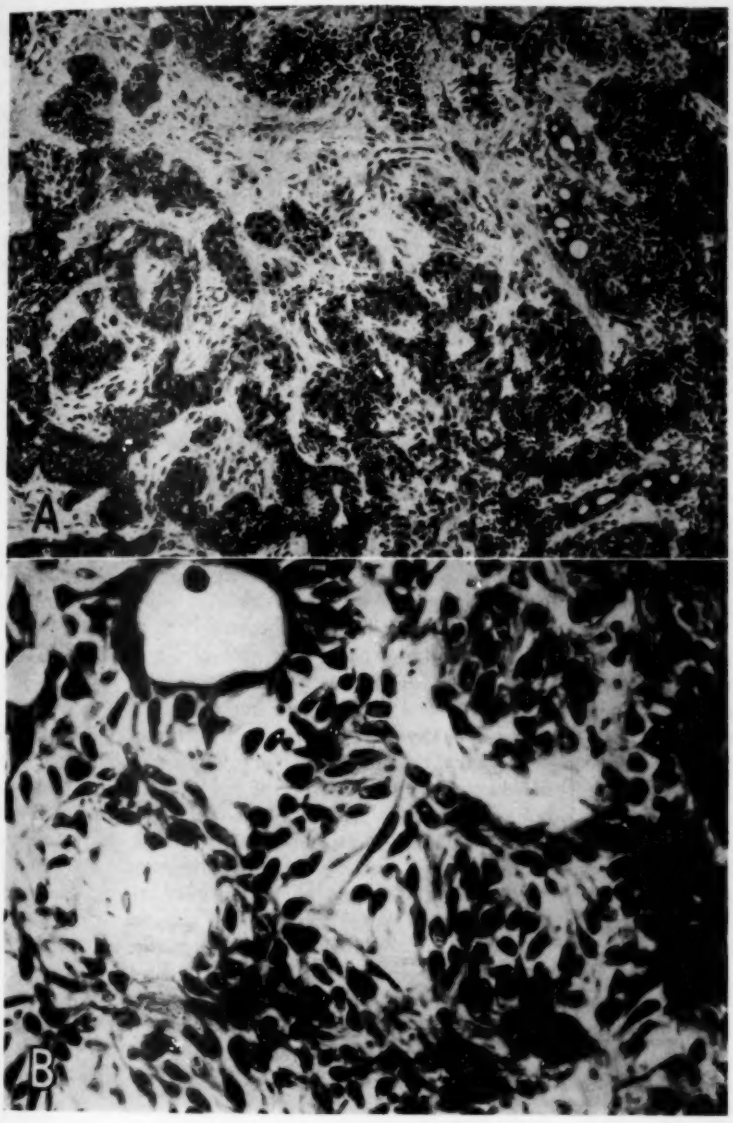

Fig. 1.—*A*, spiradenoma. Note lobular arrangement of the double-layered glands. Eosin–methylene blue; × 125. In *B*, note the proliferating outer myoepithelial layer, with the typical myoepithelial cell in the center. Eosin–methylene blue; × 450.

Microscopic Observations.—The sections showed a tumor composed of cell masses arranged in solid cords, in convoluted ductlike or glandlike formations and in dense masses without any particular arrangement (fig. 1 *A*). The tumor was surrounded by a fairly thick connective tissue capsule, which included several essentially normal eccrine sweat glands. Direct continuity between these glands and the tumor was seen in several instances. Two types of cells could be distinguished.

The first type, which was undoubtedly myoepithelial and represented the vast majority of the tumor elements, consisted of elongated, spindle-shaped cells, each with a fairly dense rod-shaped nucleus (fig. 1 *B*). On cross section the cells were triangular and the nuclei round. There was a slight degree of variation in cell and nuclear size. A few cells were twice the normal size and displayed large oval or round nuclei with evenly distributed but less dense chromatin. These cells were no longer spindle shaped but were irregularly elongated or quadrangular. Stages of transition from the spindle-shaped cell to this variety could be seen. Some multinucleated cells and rare mitoses were encountered. The cytoplasm was markedly acidophilic when stained with eosin, blue with phosphotungstic acid–hematoxylin, dark red with aniline blue and vermilion red with Masson's tetrachromate stain. Longitudinal fibrils in the cytoplasm were seen with the iron-hematoxylin and phosphotungstic acid–hematoxylin stain. These, however, could be found only in the definitely spindle-shaped cells, not in the larger atypical cells.

The ductlike and glandlike structures were generally composed of both types of cells, of which the myoepithelial ones formed the outer layer. This was frequently several rows wide. In the outermost layer the cells were not always closely spaced but were frequently separated from each other by small spaces. They connected with the basement membrane by a broad footlike cytoplasmic attachment. Where the tumor consisted of solid cords and masses, cells of the first type formed small, irregularly interwoven meshworks, which in their peripheral portions sometimes suggested a radial arrangement.

The second type of cell formed the inner layer, lining the tubular structures, but this lining was not present everywhere. These cells, which were much less numerous, were clearly epithelial and varied from flat to cuboid. They could be distinguished from the first type by their shape, their definite cellular contour, their oval to round nucleus and their finely granular cytoplasm. The last stained less intensely with eosin than the cytoplasm of the myoepithelium, orange with aniline blue and brick red with Masson's tetrachromate method. In some instances the epithelial cells showed squamous metaplasia and epithelial pearl formation, particularly where the tumor masses were without arrangement. Here the epithelial pearls formed frequently the center of the aforementioned meshworks. The lumens of the tubular structures varied somewhat in size but in general were small. In the large cell masses abortive lumens were frequently noted. Some of the lumens contained moderate amounts of granular pinkish-staining material, occasionally intermingled with degenerating desquamated epithelial cells.

Broad and fine strands of connective tissue divided the tumor into irregular lobules of varying size. The amount of stroma varied considerably in the different areas. Around the tubular structures it formed a thick basement membrane. Silver impregnation revealed a well developed system of reticulum fibrils in the stroma. Inside the basement membrane no reticulum fibrils or other connective tissue was found. With the reticulum stain, the cell boundaries of the tumor elements stood out as dark lines. This was particularly evident where the cells were densely packed and in intimate contact with one another. These lines followed the cell contours but did not connect with the basement membrane.

Fair numbers of fine elastic fibers were present. They followed the distribution of the stroma into its finest branches. Comparing the amount of elastic tissue in the tumor with that present normally around the sweat glands, a slight increase was found in some areas of the tumor.

At the periphery the stroma was fairly cellular and moderately well vascularized, while at the center it showed several areas of hyalinization. It was rich in lymphatics, many of which were distended. There was some interstitial edema.

In summary, this was a benign spiradenoma arising from the eccrine sweat glands of the cheek in which the myoepithelium was the main proliferating element.

SECOND CASE

In a 41 year old unmarried white woman, a small nodule was found incidentally in the subcutaneous tissue of the perineum. It was firm, nontender and freely movable. The presence of this mass had not been noted by the patient prior to examination.

Gross Observations.—The excised specimen consisted of a round firm mass of grayish color, measuring 8 mm. in diameter. Most of its surface was covered by a thin connective tissue capsule. The cut surfaces revealed a pearly gray, firm, trabeculated tissue.

Microscopic Observations.—The sections showed a fragment of skin with a few hair follicles and several dilated apocrine sweat glands. The corium displayed slight chronic inflammatory cell infiltrations and some recent hemorrhage, the latter without cellular response. Within the corium, and in direct continuity with the apocrine sweat glands, there was a tumor mass, the cells of which formed numerous glandular structures with multiple papillary infoldings (fig. 2 *A*). The tumor was composed of two types of cells, present in approximately equal number.

The first type consisted of elongated, spindle-shaped cells, whose nuclei were usually large and oval but occasionally were smaller and rodlike. On cross section the cells were of triangular shape and the nuclei round. Occasionally, the nucleus was twice the usual size. The rod-shaped nucleus displayed dense, uniformly distributed chromatin, usually with one nucleolus, while the chromatin in the larger type of nucleus was more loosely arranged, and there were generally two nucleoli. Mitoses were moderately frequent in these cells, sometimes as many as three in a high dry power field (fig. 2 *B*).

The cytoplasm of these cells also varied in appearance. In those with the larger, oval nuclei it stained somewhat pale pink with eosin, yellow with Van Gieson's stain and pale red with Masson's tetrachromate method. In the cells with the rodlike, denser nuclei, the cytoplasm stained more intensely red with eosin and brick red with Masson's method, while with Van Gieson's stain it maintained the yellow color. The presence of longitudinal fibrils in the cytoplasm was shown by the iron-hematoxylin stain. Sometimes they were visible with Masson's tetrachromate method.

In the glandular structures and in their infoldings the first cell type formed the outer cell layer. This varied considerably in width: in some areas it consisted of only a single row of cells, while elsewhere it was several rows in width. The cells possessed rather indistinct contours and were arranged in bundles. They lay on a basement membrane, which was broken in some instances and showed a parallel or slightly tangential course in regard to the glandular lumen.

The second cell type lined the glandular lumens and papillary projections. These cells were definitely of epithelial character. Their shape was mostly tall columnar, but sometimes cuboid. They were generally present in a single row

but occasionally showed a pseudostratified arrangement. There was considerable uniformity in cell and nuclear size. Only in rare instances were larger, slightly atypical nuclei encountered. Mitoses were rare and were found only after pro-

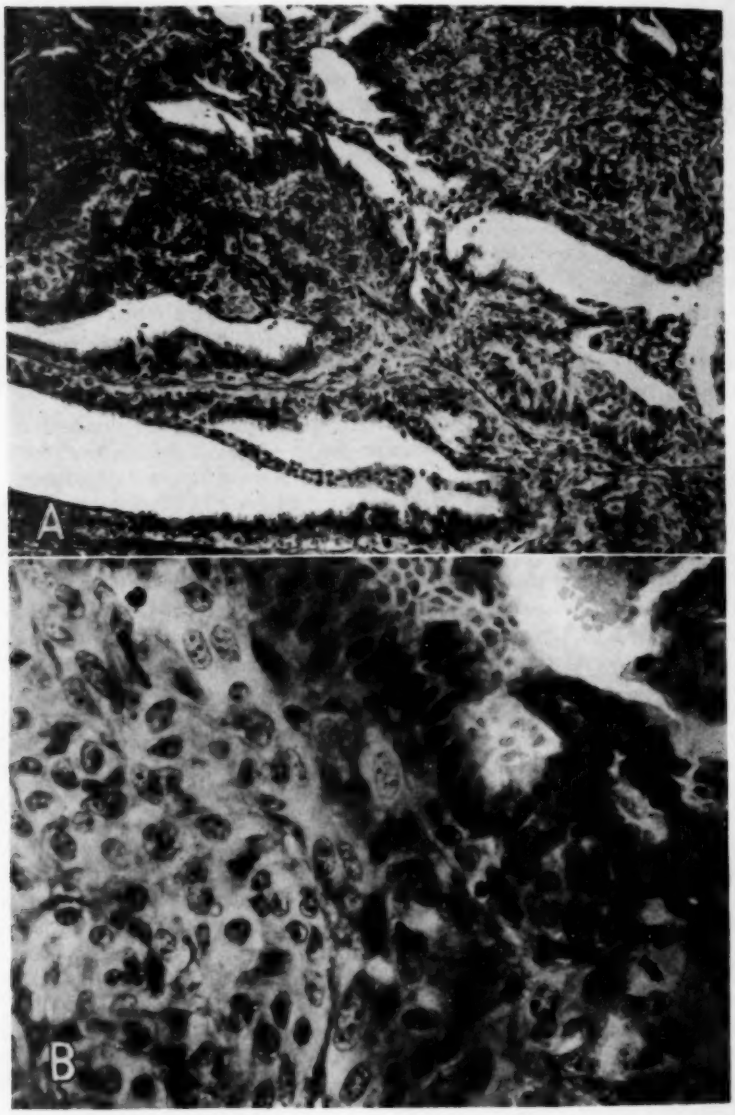

Fig. 2.—*A*, hidradenocarcinoma. Note the predominance of lighter staining, closely packed areas of myoepithelium in contrast to the darker epithelium. Hematoxylin-eosin; × 125. In *B*, note the difference between the dark staining epithelium and the lighter myoepithelium, the latter with two mitoses. Hematoxylin-eosin; × 450.

longed searching. Many of the cells displayed some secretory activity, as shown by the presence of granules in the cytoplasm above the nucleus together with beginning separation of a portion of the cell body. Some of the lumens contained small amounts of granular pinkish-staining material.

A moderate amount of stroma was present between the glandular formations. It extended with fine branches into the papillary projections and included some delicate elastic fibers. Silver impregnation showed a well developed network of reticulum fibrils, which continued into the papillary projections. No reticulum or other connective tissue was present between the tumor cells inside the basement membrane. A few capillaries were present in the stroma. The tumor was surrounded by a connective tissue capsule which merged with the corium and showed beginning invasion. Several small areas of necrosis were seen.

In summary, this was a tumor of low grade malignancy which had arisen from the apocrine sweat glands of the perineum. It may be classified as papillary hidradenocarcinoma. It was formed by approximately equal amounts of epithelium and myoepithelium, of which the latter appears to have been the more active and more malignant.

THIRD CASE

A 31 year old married white woman presented a firm, slightly tender, bluish round tumor, approximately 3 cm. in diameter, on the medial side of the base of the left great toe. The tumor was not fixed to the bone. It had grown only slightly until seven months prior to entry, when it started to increase rapidly in size and became tender.

Gross Observations.—The specimen showed an approximately round, encapsulated mass. The external surface was somewhat lobulated, of dark grayish brown color and adherent to a piece of skin measuring 2 by 1 cm. The tumor mass proper measured 2 by 3 by 3.5 cm. and was moderately soft with a few firmer areas. The cut surfaces showed a trabeculated, pearly gray tissue with a centrally located brownish area ranging from 5 mm. to 1 cm. in size. Beneath the surface were several flattened blood-filled cavities, averaging 4 mm. in size.

Microscopic Observations.—The sections showed skin with numerous eccrine sweat glands. There were no hair follicles or sebaceous glands. Within the corium was an irregularly lobulated tumor. The tumor cells were arranged in small to large, mostly solid masses and cords, which were densely packed in some areas and loosely scattered elsewhere. Occasionally, an alveolar arrangement was encountered (fig. 3*A*). Direct continuity between the eccrine sweat glands and the tumor can be seen in several instances. Two types of cells were present in the tumor.

The first type, which by far predominated, showed considerable variation in the size and appearance of its elements. The cells were generally large and showed well defined contours. Most of the cells were of round to oval shape on cross section but more or less elongated when cut longitudinally, although occasionally they were spindle shaped. The nuclei were generally large, of vesicular appearance and of round or oval shape, averaging one nucleolus. The nuclear membrane was distinct. Multinucleated cells, with from two to eight nuclei, were frequent, but mitoses were rare (fig. 3*A*).

Large, atypical cells were numerous, some of which reached three to four times the dimensions of the other elements. They were occasionally of fusiform shape with dense rodlike nuclei (fig. 3*B*). Others displayed irregularly shaped nuclei with two to four nucleoli. The nuclei were placed somewhat eccentrically within the cell bodies. The cells displayed large amounts of cytoplasm, which stained

intensely red with eosin, blue with phosphotungstic acid–hematoxylin, dark red with aniline blue and vermilion red with Masson's tetrachromate method. It was generally of homogeneous appearance, with some vacuolation immediately beneath

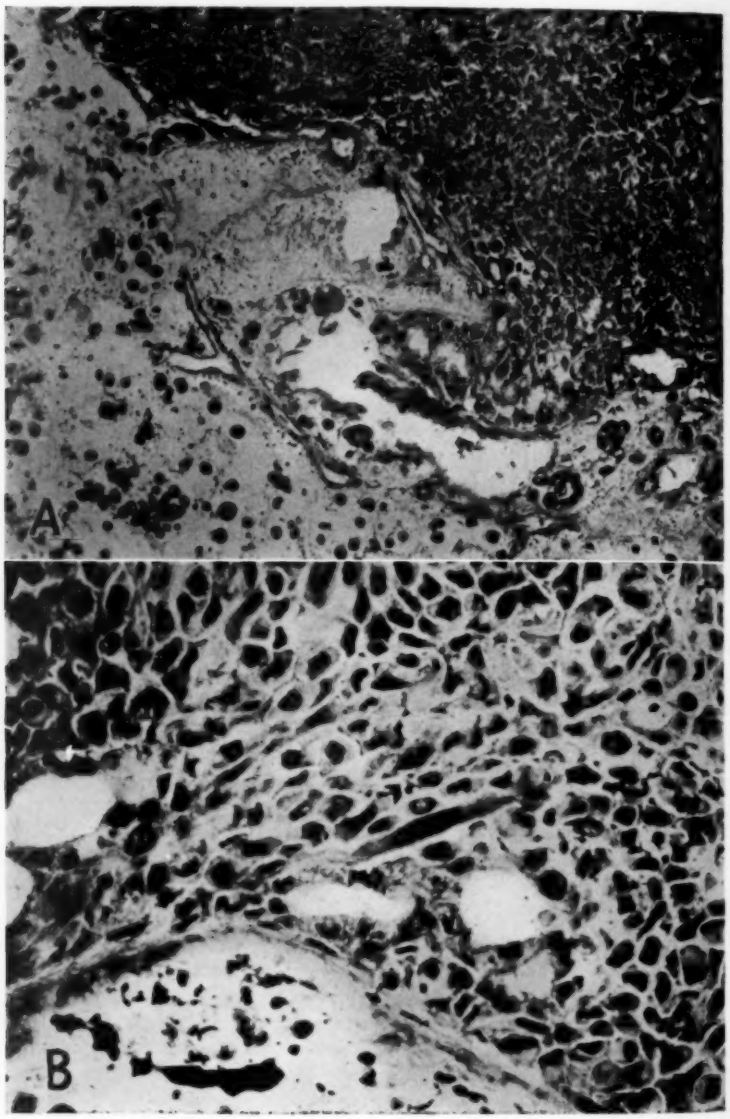

Fig. 3.—*A*, malignant myoepithelioma. Note the multinucleated tumor cells in the right upper corner and the alveolar arrangement in the center. Hematoxylin-eosin; × 125. In *B*, note in center the large atypical myoepithelial cell surrounded by proliferating myoepithelium. Phosphotungstic acid–hematoxylin; × 450.

the cell membrane. The phosphotungstic acid–hematoxylin and iron-hematoxylin stains revealed longitudinal cytoplasmic fibrils in the less anaplastic cells.

The elements of this first type were generally without any particular architectural plan, but in some areas they were arranged in bundles. At the periphery of the tumor masses they displayed occasionally a suggestion of loose palisading with small spaces between the single elements. Where the tumor presented an alveolar arrangement, these cells formed the outer cell layer, sometimes several cells in width.

Relatively few cells represented the second type. They lined the small gland-like structures, where they were present in a single inner row of cells. The latter were definitely of epithelial character and of cuboid or flattened shape. There was no variation in cell or nuclear size. No mitoses were present. The finely granular cytoplasm showed occasional small secretion vacuoles. It stained as described before. The lumens were empty but were frequently distorted or obliterated.

Small to large strands of connective tissue divided the tumor into irregular lobules of varying size. In many instances the stroma surrounded the tumor lobules in basement membrane–like fashion. Fragmentation and penetration of this membrane by the tumor were noted in many instances. A well developed network of reticulum fibrils was present. These were always located peripherally to the outer cell layer and were never interposed between this and the epithelium.

No new formation of reticulum by the tumor cells was noted anywhere. A fairly well developed network of elastic fibers, sometimes finely branching, was present in some areas, while in others it was lacking. The stroma displayed hyalinization, although in the center of the tumor areas of mature fibrous cartilage were encountered.

Small and medium-sized blood vessels were moderately frequent. Fairly large areas of recent hemorrhage, sometimes associated with necrosis and fibrinoid degeneration, were found. These together with the marked edema present in other areas caused a moth-eaten appearance.

Toward the epidermis the tumor was surrounded by a thick capsule of dense hyaline connective tissue. Invasion of this capsule by the tumor was noted in several places.

In conclusion, this was a malignant tumor arising from the eccrine sweat glands of the foot. The main tumor element was represented by neoplastic, markedly atypical myoepithelium. The picture resembled in some areas that of leiomyosarcoma.

COMMENT

Ten sweat gland tumors of both the benign and the malignant variety were studied. These had arisen in different parts of the body and included various types. In 3 of the 10 tumors it was found that the myoepithelium played the leading part in the formation of the tumor. In 2 of the 3 tumors it was by far the predominant tumor element. In all 3 it was more active than the epithelium forming the second cellular constituent of the tumor.

One of these tumors, which presented the picture of spiradenoma, was benign. It had arisen from the eccrine sweat glands of the cheek. The second tumor had originated from the apocrine sweat glands of the perineum and was of low grade malignancy. It might be termed papil-

lary hidradenocarcinoma. The third tumor was derived from the eccrine sweat glands of the foot. It was of essentially solid type and showed a more advanced grade of malignancy. It is difficult to name this tumor, for which at present a descriptive term, such as "malignant myoepithelioma," might be used.

The ages of the 3 patients, who were all of the female sex, were 10 5/12, 31 and 41 years. The first 2 patients had their tumors removed too recently for any follow-up examination. The third patient was seen after seven years and did not show recurrence or metastases. The number of cases is too small to permit any conclusions in regard to the sex and age incidence, but these are probably the same as in cases of sweat gland tumors in general. The same applies to the prognosis, which seems to be rather favorable, even when the tumor is definitely malignant.

The morphologic character of the myoepithelium undergoing neoplastic proliferation varied somewhat in the 3 cases. In the first case, in which the double-layered arrangement of the epithelium and myoepithelium was, in general, well preserved, the myoepithelium formed the outer cell layer and displayed the typical appearance of smooth muscle fibers, although some anaplastic elements were present. The latter were larger and ranged from irregularly elongated to oval or round. The longitudinal fibrils in the cytoplasm tended to be absent. The nuclei, instead of being rodlike, were oval or round, while the chromatin was less dense and sometimes vesicular. Multinucleated cells appeared, and a few mitoses were found. The typical arrangement into two layers was lost in many areas. In the second case, the cells were generally of the usual spindle shape, and the longitudinal fibrils of the cytoplasm were preserved in many instances. The nuclei had undergone the described changes, and mitoses were fairly frequent. The epithelium participated in the neoplastic proliferation, although to a lesser extent than the myoepithelium. The usual relationship between the two cell types was preserved, but there was more marked proliferation of the myoepithelium, which was sometimes several layers in depth. In the third case, marked pleomorphism in cell and nuclear size was found. Only a few cells of unaltered appearance were present at the periphery of the tumor masses. Most of the cells were markedly anaplastic. Multinucleated and atypical cells were numerous, but mitoses were rare. The cytoplasm began to show vacuolation. There were no longitudinal fibrils. Except for occasional small alveolar formations lined by nonproliferating epithelium, the arrangement of the neoplastic myoepithelial elements was disorderly and without any architectural plan. The picture in some areas resembled that of leiomyosarcoma.

In the first and third cases, a fair amount of supporting stroma was present. It was less developed in the second case. Sometimes the

stroma displayed areas of hyalinization. In the third case some fibrous cartilage was found within the hyalinized stroma in the center of the tumor. There was no evidence that the cartilage or the reticulum fibrils were derived from the tumor cells, as was observed by Hamperl.[6] Furthermore, contrary to the finding of Loos,[10] elastic fibers were present in the tumors.

The classification of these tumors presented difficulties. They were predominantly composed of cells which displayed all the morphologic and staining characteristics of smooth muscle fibers. The mesodermal origin of smooth muscle in general is universally accepted, while the ectodermal derivation of the myoepithelium appears to be unanimously agreed on by the anatomists and histologists who have studied this problem.

Hamperl[6] concluded from his own and other findings that the distinction between epithelium and mesenchyma, on which the present tumor classifications are based, is not always as sharply defined as one has been led to assume. He pointed out that there is probably a borderline region between ectoderm and mesoderm where the cells are endowed with the potentialities of both tissues and are capable of developing into elements of epithelial as well as mesenchymal character. He expressed the belief that it is from this borderline region that the myoepithelial tumors arise.

The more recent developments in embryology as reviewed by Streeter[11] tend to abolish the concept of different germ layers in favor of a single totipotential primitive tissue, and would seem to point in a somewhat similar direction.

Weiner,[12] in his paper on extramammary Paget's disease, suggested that the skin lesion is caused by intraepidermal metastasis from underlying carcinoma of the apocrine sweat glands. Bearing in mind that the myoepithelium in neoplastic proliferation may assume a markedly atypical appearance, a study of these elements in Paget's disease in general, as well as particularly in regard to a possible relation to the so-called Paget cells proper, could be of interest.

SUMMARY

Three cases of sweat gland tumor are described in which the myoepithelium had undergone neoplastic proliferation. One of the tumors was benign, while the remaining 2 were malignant. In 1 case both the myoepithelium and the epithelium appeared to be involved in the neoplastic process, while in the 2 other instances the myoepithelium represented the main tumor element. The difficulty in classifying these tumors is pointed out.

11. Streeter, G. L.: Anat. Rec. **64**:48, 1936.
12. Weiner, H. A.: Am. J. Cancer **31**:373, 1937.

CONGENITAL BICUSPID PULMONARY VALVES

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This report comprises a study of 9 cases of congenital bicuspid pulmonary valve. Eight of these were encountered in a series of 3,600 consecutive autopsies at the Institute of Pathology of the University Hospitals of Cleveland. An additional case, recently studied at the City Hospital, Cleveland, is described because of its especial interest.

REPORT OF CASES

Congenital bicuspid pulmonary valves can be conveniently subdivided into three groups as follows: (1) those in hearts with the tetralogy of Fallot; (2) those in hearts with anomalies other than the tetralogy of Fallot; (3) those in hearts with no other congenital anomalies.

Group 1. Cases in Hearts with the Tetralogy of Fallot.

1. A 17 year old white girl died of acute purulent meningitis. A diagnosis of congenital heart disease was made during life.

The heart weighed 230 Gm. and revealed the tetralogy of Fallot: pulmonary stenosis, dextroposition of the aorta, patent interventricular septum and hypertrophy of the right ventricle. There was thickening of the tricuspid valve, which showed several small fresh nodular verrucae along the line of closure. The pulmonary conus was markedly constricted at its lower bulbar orifice. The pulmonary valve was composed of two thin delicate cusps of unequal size. The larger measured 2 cm. in length and was situated to the left and posteriorly, while the smaller measured 1.5 cm. in length and was placed to the right and anteriorly (figs. 2 [case 1] and 3*A* [gross specimen]). In the sinus of Valsalva of the larger cusp was a small flat fibrous band, situated just above the attachment of the cusp and measuring 2 by 1 by 0.5 mm. (fig. 3*A*, *a*). These dimensions represent the length (from the point of origin on the pulmonary wall of the sinus of Valsalva to the insertion in the base of the cusp), the width and the height (from the ring of the valve to the free margin). There was no stenosis of the valve, and the commissures showed no abnormality.

Serial transverse microscopic sections[1] revealed that the entire fibrous band consisted of compact collagenous and hyalinized connective tissue, which was slightly cellular and showed no vascularity or exudate (fig. 3*A* [transverse

From the Institute of Pathology, Western Reserve University and the University Hospitals of Cleveland.

1. Microscopic sections were studied with the hematoxylin and eosin stain, with the Weigert technic for elastic tissue and with the combined Weigert and Van Gieson methods for elastic and connective tissue.

section]). No elastica was present. Proximally the junction of the connective tissue and the adjacent media of the pulmonary artery occurred on a horizontal plane.

Microscopic sections of the tricuspid valve revealed chronic and subacute valvulitis. Small fibrinous vegetations were attached to the line of closure. Sections of the pulmonary cusps showed no significant change.

2. A 26 year old white man died of cardiac failure. The clinical diagnosis was congenital heart disease, probably the tetralogy of Fallot.

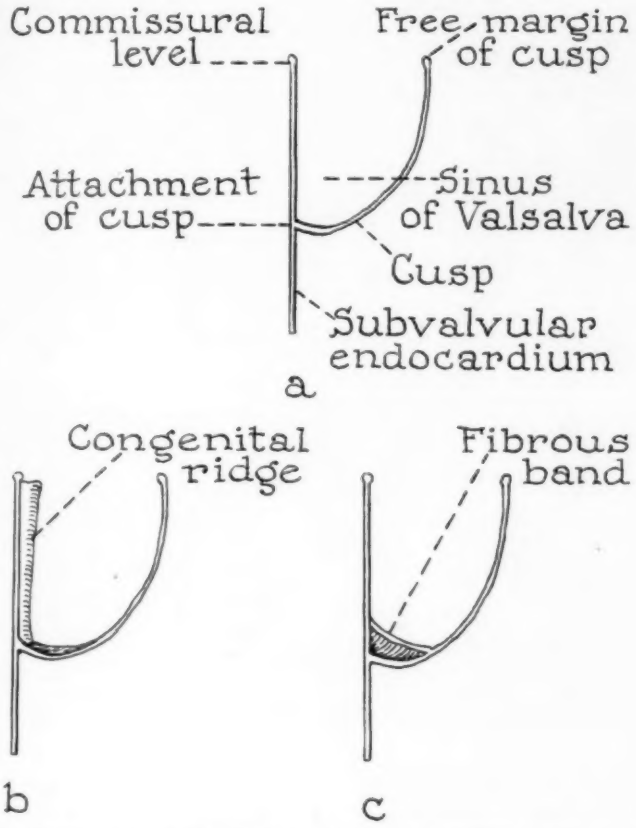

Fig. 1.—Drawings of longitudinal sections of pulmonary valves to show the relation of the congenital ridge and of the fibrous band to the sinus of Valsalva: (*a*) normal value; (*b*) congenital ridge; (*c*) fibrous band.

The heart weighed 480 Gm. and revealed the tetralogy of Fallot. Along the line of closure of the tricuspid valve were several small fresh verrucae. An additional anomaly was a bicuspid pulmonary valve. The two cusps were thin, delicate and of equal size, each measuring 1.8 cm. in length. The circumference of the aortic valve was 5 cm. One pulmonary cusp was situated to the right and posteriorly, while the other was placed to the left and anteriorly (figs. 2 [case 2] and 3*B* [gross specimen]). The former revealed a small firm fibrous band in the

depth of its sinus of Valsalva (fig. 3 *B, a*). This was centrally located, measured 3 by 2 by 2 mm., began only 2 mm. above the attachment of the cusp and passed obliquely across it to insert into the base of the cusp. There was no suggestion of a congenital ridge in the upper portion of the sinus of Valsalva. The commissures of the valve showed no abnormality.

A longitudinal microscopic section revealed that the entire fibrous band was composed of relatively acellular collagenous connective tissue (fig. 3 *B* [longi-

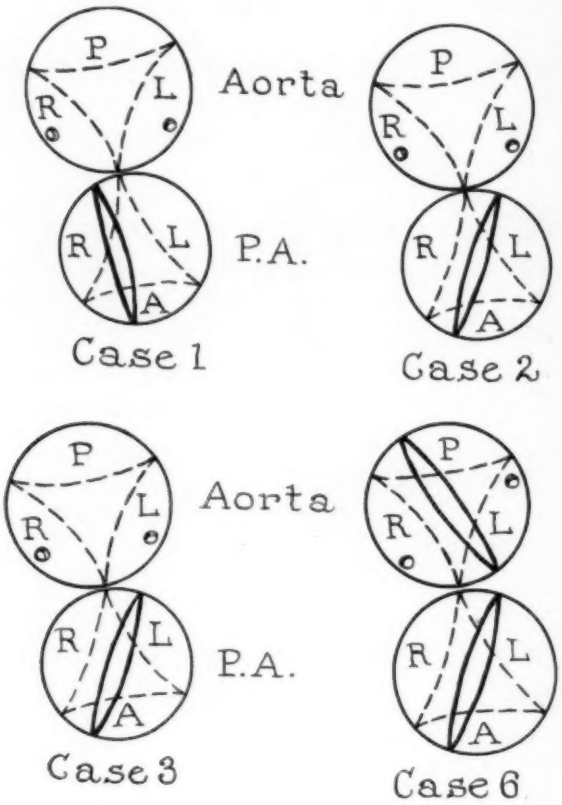

Fig. 2.—These diagrams illustrate the position of the cusps (shown in heavy lines) in the bicuspid pulmonary valves. The broken lines indicate the normal positions of the pulmonary and aortic cusps. The pulmonary artery (*P. A.*) is situated to the left and anteriorly and the aorta to the right and posteriorly.

Case 1. A 17 year old white girl with the tetralogy of Fallot and a congenital bicuspid pulmonary valve. The larger cusp is situated to the left and posteriorly and the smaller to the right and anteriorly.

Case 2. A 26 year old white man with the tetralogy of Fallot and a congenital bicuspid pulmonary valve. The two cusps are of equal size.

Case 3. A 24 year old white woman with the tetralogy of Fallot and a congenital bicuspid pulmonary valve. One pulmonary cusp was situated posteriorly and slightly to the right, while the other was anterior and to the left.

Case 6. A 2 month old white girl with congenital bicuspid pulmonary and aortic valves.

tudinal section]). There was no vascularity or inflammation. Proximally, the connective tissue arose anterior to the adjacent terminal portion of the media of the pulmonary artery.

The tricuspid valve was the seat of subacute valvulitis. The pulmonary cusps showed no abnormality.

3. A 24 year old white woman had a clinical diagnosis of congenital heart disease, probably the tetralogy of Fallot. Death was due to cardiac failure.

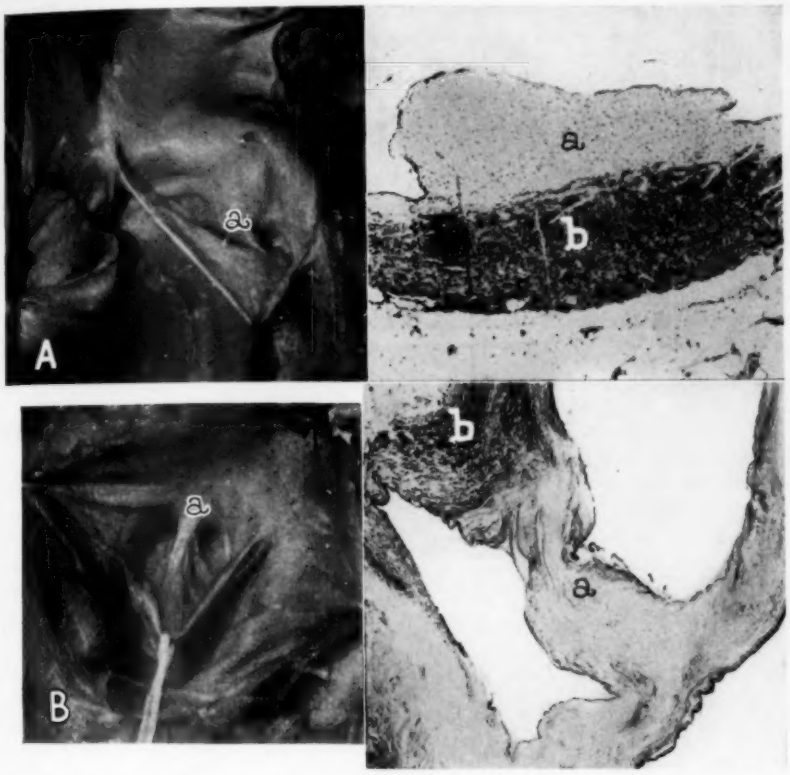

Fig. 3.—In *A* (case 1) there is a small fibrous band (*a*) in the sinus of Valsalva of the larger of the two pulmonary cusps. The transverse section of the fibrous band shows that it consists entirely of compact connective tissue (*a*), which rests on myocardium (*b*). Hematoxylin and eosin stain; × 100.

In *B* (case 2), the posterior pulmonary cusp is retracted to show the fibrous band (*a*) situated at the bottom of the sinus of Valsalva. The longitudinal section of the fibrous band shows dense collagenous connective tissue (*a*), which proximally lies anterior to the adjacent terminal media (*b*) of the pulmonary artery. Weigert elastic stain; × 27.

The heart weighed 250 Gm. and showed the tetralogy of Fallot. In addition, the pulmonary valve was bicuspid, consisting of two thin delicate cusps of equal size, each measuring 2 cm. in length. The circumference of the aortic valve was 5.5 cm. One pulmonary cusp was situated posteriorly and slightly to the right,

while the other was anterior and to the left (fig. 2 [case 3]). The posterior cusp revealed two small delicate fibrous bands deep in the sinus of Valsalva, one in the center and the other in the left lateral third of the sinus. Both bridged the attachment of the cusp, passing from the base of the pulmonary wall of the sinus of Valsalva to the base of the cusp.

Transverse microscopic sections through the fibrous bands revealed that the outer portion was composed of compact collagenous tissue, while the inner part consisted of a looser type of connective tissue (fig. 4 *A*). There was no elastica. No inflammation or vascularity was present.

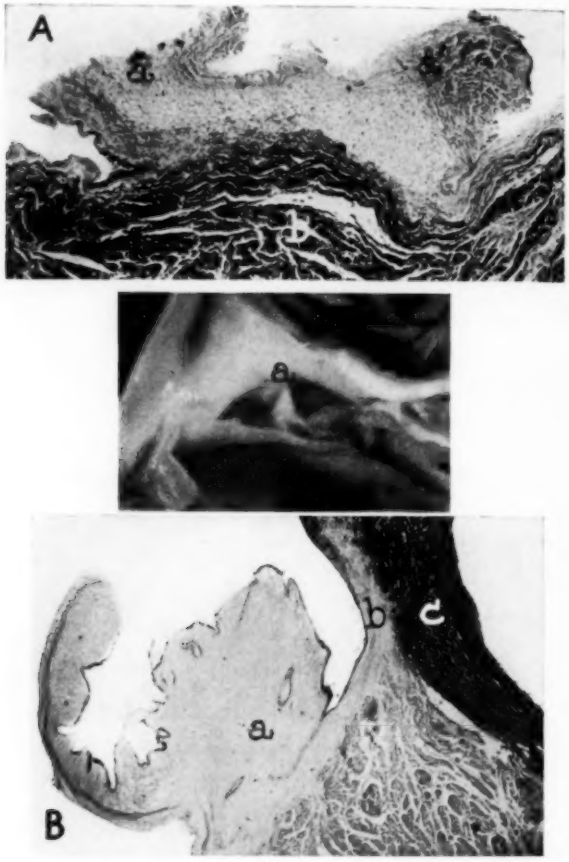

Fig. 4.—*A* (case 3), transverse section of the fibrous bands in the sinus of Valsalva of the posterior cusp of the bicuspid pulmonary valve. The bands (*a* and *a'*) consist of avascular connective tissue, which rests on myocardium (*b*). Hematoxylin and eosin stain; × 40.

In *B* (case 6), upper part, the right pulmonary cusp reveals a centrally located robust wedge of fibrous tissue (*a*) in the bottom of the sinus of Valsalva. The longitudinal section of the fibrous wedge shows that it consists of compact connective tissue (*a*). Proximally there is an upward extension of fibrous tissue (*b*) which lies anterior to the media (*c*) of the pulmonary artery. Weigert elastic stain; × 30.

Sections of the pulmonary cusps showed no abnormality.

Group 2. Cases in Hearts with Anomalies Other Than the Tetralogy of Fallot.

4. A 1 day old white boy died of acute purulent meningitis complicating meningomyelocele.

The heart weighed 35 Gm.[2] The pulmonary and aortic valves were both of the bicuspid type. Each measured 2.5 cm. in circumference and consisted of two well formed, slightly thickened cusps, equal in length. Neither of the cusps was subdivided by a congenital ridge and neither revealed a fibrous band in the sinus of Valsalva.

No microscopic sections of the pulmonary valve were made.

5. A 2 year old Negro boy died of bronchopneumonia. Incidental findings at autopsy were a congenital bicuspid pulmonary valve and a widely patent foramen ovale. The pulmonary valve consisted of two entirely normal thin delicate cusps. One was situated to the right and measured 1 cm. in length, while the other was situated to the left and measured 1.5 cm. in length. The commissures showed no abnormality. The aortic valve was normal, and its ring circumference was 3.8 cm.

No microscopic sections of the pulmonary valve were made.

6. A white girl 2 months of age died of bronchopneumonia.

The heart revealed a patent interventricular septum and bicuspid pulmonary and aortic valves. The circumference of the pulmonary valve was 3.2 cm. and that of the aortic valve 2.7 cm.

The pulmonary valve was composed of two slightly thickened cusps of equal size. One was placed to the right and posteriorly and the other to the left and anteriorly (figs. 2 [case 6] and 4 *B* [gross specimen]). The former revealed a centrally located robust wedge of fibrous tissue in the bottom of the sinus of Valsalva (fig. 4 *B*, *a*). This measured 3 by 1 by 2 mm. and passed directly forward from the pulmonary wall of the sinus to the base of the cusp just beyond the latter's site of attachment.

The aortic valve consisted of two delicate cusps of equal size, one being situated to the right and anteriorly (right coronary cusp) and the other to the left and posteriorly (left coronary cusp). The latter revealed a tiny centrally located fibrous band deep in its sinus of Valsalva, which passed across the junction of the cusp and sinus.

A longitudinal microscopic section showed that the fibrous band in the pulmonary valve was composed of moderately cellular connective tissue, which was largely compact but revealed a few areas of looser arrangement (fig. 4 *B*, *a*, *b*). There were several capillaries at the base, but no exudate was present. Proximally, the connective tissue was situated anterior to the media of the pulmonary artery which passed down behind it throughout its length (fig. 4 *B*, *c*).

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2. The expected weight of the heart would be 23 Gm. according to R. Roessle and F. Roulet (Mass und Zahl in der Pathologie, in Aschoff, L.; Elias, H.; Sternberg, C., and Wenckebach, K. F.: Pathologie und Klinik, in Einzeldarstellungen, Berlin, Julius Springer, 1932, vol. 5), 17 Gm. according to T. M. Copoletta and S. B. Wolbach (Body Length and Organ Weight of Infants and Children, Am. J. Path. **9**:55, 1933) and 24 Gm. according to C. F. Craig (Laboratory Methods of the U. S. Army, ed. 3, Philadelphia, Lea & Febiger, 1929).

Microscopic sections of the pulmonary cusps showed fibrous thickening of the free portions.

7. A white boy 8 months of age had clinical diagnoses of congenital heart disease and bronchopneumonia.[3]

The heart showed the following congenital anomalies: cor biatriatum triloculare, complete transposition of the great arterial trunks and a bicuspid pulmonary valve. The ring circumferences of the aortic and pulmonary valves each measured 3 cm. The two pulmonary cusps were thick and fleshy; one was situated to the right and slightly posteriorly and measured 1.8 cm. in length, while the other was placed to the left and slightly anteriorly and measured

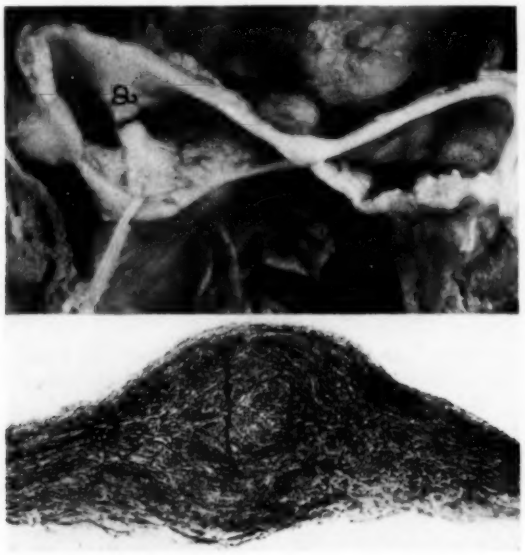

Fig. 5 (case 7).—Both pulmonary cusps are thick and fleshy. The larger cusp reveals a congenital ridge (*a*) in the sinus of Valsalva. The transverse section of the proximal portion of the congenital ridge shows that it consists largely of elastic fibers derived from the pulmonary media, and in the center these have a whorled pattern. The annulus fibrosus is not seen at this level. Weigert elastic stain; × 40.

1.2 cm. in diameter (fig. 5 [gross specimen]). The larger cusp was evenly subdivided by a typical congenital ridge, measuring 5 by 1.5 by 0.5 mm. (fig. 5*a*). This consisted of a slight uniform longitudinal hemicylindric elevation of the pulmonary artery extending from the upper level of the sinus of Valsalva (level of the commissure) to the bottom of the sinus. At its termination it gave rise to a thin subsidiary fibrous fold which continued directly forward almost to the free margin of the cusp.

Serial transverse microscopic sections showed that the proximal portion of the ridge was composed essentially of bundles of elastic tissue which passed uninter-

3. This case was studied at the City Hospital, Cleveland.

ruptedly across the ridge in a symmetric manner and were continuous laterally with the media of the pulmonary artery (fig. 5, lower part). Centrally, the elastica revealed a whorled pattern. As the elastic tissue thinned out distally, it overlapped the adjacent annulus fibrosus of the ring of the cusp both superficially and deeply. There was no vascularity or inflammation.

Microscopically, there was fibrous thickening of the pulmonary cusps.

Group 3. Cases in Which the Bicuspid Pulmonary Valve is the Only Congenital Deformity of the Heart.

8. A 58 year old Negro man had clinical diagnoses of hypertension and arteriosclerosis and died in uremia. Except for moderate enlargement of the heart he was found normal on physical examination.

The heart weighed 450 Gm. The only pathologic changes consisted of hypertrophy and dilatation, and there was a congenital bicuspid pulmonary valve. The latter was composed of two normal thin delicate cusps of equal length, one of which was situated anteriorly and the other posteriorly. The commissures showed no abnormality. Neither cusp revealed any change in the sinus of Valsalva.

No microscopic sections of the pulmonary valve were made.

9. A 56 year old white man died of bronchopneumonia. The clinical diagnoses were generalized arteriosclerosis and arteriosclerotic heart disease. Physical examination revealed enlargement of the heart, but there were no cardiac murmurs.

The heart was the seat of hypertrophy and dilatation and weighed 600 Gm. The only other pathologic finding was a congenital bicuspid pulmonary valve. This consisted of one large cusp, situated posteriorly and evidently representing a combined right and left posterior cusp, and a smaller cusp, situated anteriorly. In the center and extreme base of the sinus of Valsalva of the larger cusp there was a narrow, slightly elevated band of fibrous tissue which passed from the pulmonary wall of the sinus to the cusp just beyond the latter's site of attachment.

No microscopic sections of the pulmonary valve were made.

SUMMARY OF NINE CASES OF CONGENITAL BICUSPID PULMONARY VALVE

The data with regard to age, sex and color, together with the clinical diagnoses and the associated cardiac and extracardiac anomalies, are summarized in table 1.

In 3 cases the bicuspid pulmonary valve occurred in an infant, and in 1 case, in a 2 year old boy; in 3 cases it was observed in a young adult with the tetralogy of Fallot, and in 2, in an elderly adult in whom the deformity was an isolated cardiac anomaly.

A clinical diagnosis of congenital heart disease was made in 4 cases, in 3 of which the associated condition was found to be the tetralogy of Fallot; in 5 cases the bicuspid pulmonary valve was an entirely incidental finding at autopsy.

The position of the two pulmonary cusps varied, and there was no fixed pattern. Usually, however, one cusp was placed to the right and the other to the left, and either was anterior or posterior to a varying degree.

The two pulmonary cusps were equal in size in 5 cases and unequal in 4. In 7 of the 9 cases the cusps were thin and delicate, while in 2 cases they showed slight diffuse thickening. None of the valves revealed

TABLE 1.—*Summary of Nine Cases of Congenital Bicuspid Pulmonary Valve*

Case	Age, Sex and Color	Clinical Diagnosis	Cause of Death	Other Cardiac Anomalies	Extracardiac Anomalies
1	17 yr. Female White	Acute purulent meningitis; congenital heart disease	Meningitis	Tetralogy of Fallot	None
2	26 yr. Male White	Congenital heart disease	Cardiac failure	Tetralogy of Fallot	None
3	24 yr. Female White	Congenital heart disease; right hemiplegia	Cardiac failure	Tetralogy of Fallot	None
4	1 day Male White	Meningocele; acute suppurative meningitis	Meningitis	Bicuspid aortic valve	Meningomyelocele; talipes equinovarus; supernumerary tragus
5	2 yr. Male Negro	Bronchopneumonia	Bronchopneumonia	Widely patent foramen ovale	None
6	2 mo. Female White	No diagnosis made	Bronchopneumonia	Patent interventricular septum; bicuspid aortic valve	Meckel's diverticulum; heterotopic pancreas in jejunum
7	8 mo. Male White	Congenital heart disease	Bronchopneumonia and cardiac failure	Cor biatriatum triloculare; complete transposition of the great vessels	None
8	58 yr. Male Negro	Hypertension and arteriosclerosis; nephrosclerosis	Uremia	None	Heterotopic pancreas in stomach
9	56 yr. Male White	Arteriosclerotic heart disease; bronchopneumonia	Bronchopneumonia	None	None

TABLE 2.—*Circumferences of the Pulmonary and Aortic Valves in Nine Cases of Congenital Bicuspid Pulmonary Valve*

Case	Circumference of Pulmonary Valve, Cm.	Circumference of Aortic Valve, Cm.
1	3.5	6.0
2	3.6	5.0
3	4.0	5.5
4	2.5	2.5
5	2.5	3.8
6	3.2	2.7
7	3.0	3.0
8	7.5	7.0
9	8.0	7.2

gross or microscopic evidence of inflammation or of superimposed calcification. Rudimentary cusps were not encountered.

Three of the 9 bicuspid valves were of the simple type consisting of two well formed cusps of equal size. In 5 valves one of the two cusps revealed a small fibrous band centrally located in the bottom of the

sinus of Valsalva. Two such valves had cusps of unequal size, and the fibrous band occurred in the larger cusp in each valve. In 1 valve (case 3) two fibrous bands were present, one in the center and the other in the lateral third of the sinus of Valsalva. Only 1 valve (case 7) revealed a conjoined cusp subdivided by a typical congenital ridge.

A comparison of the circumference of the pulmonary with that of the aortic valve is shown in table 2. The pulmonary valve was smaller in 4 cases and equal or slightly larger than the aortic valve in 5 instances. The most marked differences were in the 3 cases in which autopsy revealed the tetralogy of Fallot. These showed hypoplasia of the pulmonary artery, and the pulmonary valve was distinctly smaller than the aortic valve.

COMMENT

Congenital bicuspid pulmonary valves can be discussed conveniently with respect to frequency, associated cardiac anomalies, morphologic aspects, embryologic development and clinical significance.

TABLE 3.—*Frequency of Congenital Cardiac Anomalies Among 3,600 Consecutive Autopsies at the Institute of Pathology, University Hospitals of Cleveland (1930-1940)*

Anomaly	Cases
Bicuspid aortic valve	20
Coarctation of aorta	17
Patent interventricular septum	16
Transposition of the great vessels	15
Patent ductus arteriosus	11
Pulmonary stenosis	8
Bicuspid pulmonary valve	7
Pulmonary valve with four cusps	6

Frequency.—Among the 3,600 consecutive autopsies reviewed in this study, there were 68 which disclosed one or more congenital deformities of the heart. Table 3 shows the most common of these anomalies in the order of their frequency. The anomalies were tabulated regardless of whether or not they occurred alone or in combination with other anomalies, or were of primary or secondary importance.[4] There were 8 bicuspid pulmonary valves, an incidence of 0.22 per cent. On the other hand, there were 20 congenital bicuspid aortic valves (0.55 per cent), this being the most common cardiac anomaly. Coarctation of the aorta and patent interventricular septum were next in the order of frequency. The pulmonary valve was composed of four cusps in 6 cases, and there were no cases of aortic valve with four cusps.

4. Instances of anatomically patent foramen ovale were excluded, as were those of variations in the origin and distribution of the coronary arteries. Instances of patent ductus arteriosus were excluded if the infant was 2 months or less of age unless the patent duct was unusually large.

Comparable data on the relative frequency of variations in the number of cusps of the semilunar valves are shown in table 4. This table gives the total number of consecutive autopsies reviewed, for the most part recently, at various institutions and the number among these in which pulmonary and aortic valves with two and also with four cusps were observed. The bicuspid aortic valve is the most frequent condition (68 examples in over 44,252 autopsies) while the aortic valve with four cusps is the least frequent and is in fact rare (7 examples).

TABLE 4.—*Relative Frequency of Congenital Anomalies of the Pulmonary and Aortic Valves Found at Autopsy*

Year of Publication	Institution and Observer	Autopsies	Congenital Bicuspid Pulmonary Valves	Pulmonary Valves with Four Cusps	Congenital Bicuspid Aortic Valves	Aortic Valves with Four Cusps
1918	Amsterdam Institute of Pathology (de Vries [7])	3,600	3	9	12	1
1923	Northwestern University (Simonds [11])	2,000	0	1	1	0
1936	Philadelphia General (Szypulski, J. T.: J. Tech. Methods **17**: 119, 1937)	7,500	3	2	4	3
1936	Johns Hopkins (Leech, C. B.: J. Tech. Methods **15**: 101, 1936)	13,115	1	..	3	..
1936	University of Pennsylvania (Rannels, H. W., and Propst, J. H.: J. Tech. Methods **17**: 113, 1937)	4,255	1	1	5	0
1936	Willard Parker Hospital and Central Neurological Hospital (Dolgopol, V. B.: J. Tech. Methods **16**: 96, 1936)	1,082*	1	3	0	0
1936	Massachusetts General (McGinn, S.: J. Tech. Methods **16**: 98, 1936)	7,500	1	3	10	0
1938	University of Minnesota (Ingham, D. W.: Ann. Int. Med. **12**: 609, 1938)	Not stated	3	15	9	3
1938	New York (Jacobius, H. L., and Moore, R. A.: J. Tech. Methods **18**: 133, 1938)	1,600	2	1	4	0
1941	Institute of Pathology, University Hospitals of Cleveland	3,600	8	6	20	0

* The autopsies in this institution are limited to children with contagious diseases.

Bicuspid pulmonary valve (23 examples) and the pulmonary valve with four cusps (41 examples) lie somewhere in between in comparative incidence, and the latter is apparently encountered with slightly greater frequency than the bicuspid lesion. Two cases of pulmonary valve with five cusps and a case of an aortic valve with five cusps have been recorded.[5]

Abbott's [6] tabulation, in 1932, of the anomalies in 1,000 cases of congenital heart disease collected from the literature includes a total incidence of 125 pulmonary and aortic valves with two or four cusps.

5. Dilg, J.: Virchows Arch. f. path. Anat. **91**:193, 1883.

6. Abbott, M. E.: Atlas of Congenital Cardiac Disease, New York, American Heart Association, 1936.

There were 43 so-called primary anomalies of this type and 82 anomalies complicating other defects as shown in table 5.

Again the bicuspid aortic valve is comparatively frequent, while the aortic valve with four cusps is rare, i. e., only 4 instances in 1,000 cases of congenital cardiac disease. The bicuspid pulmonary valve appears to be much more frequent than the pulmonary valve with four cusps, but this is probably due to the fact that a greater number of bicuspid valves was reported in the literature because of the association of this anomaly with more serious cardiac anomalies. It is of interest to note that in Abbott's data the bicuspid pulmonary valve is recorded as a primary deformity in only 1 case, as compared with 30 instances in which it was secondary to some other deformity of the heart.

Associated Cardiac Anomalies.—Although the bicuspid pulmonary valve may be an isolated finding, it is usually associated with other congenital deformities of the heart. These frequently consist of serious changes, such as patent interventricular septum, pulmonary stenosis

TABLE 5.—*Frequency of Anomalies of the Semilunar Valves in One Thousand Cases of Congenital Heart Disease (Abbott)*

	Bicuspid Pulmonary Valves	Pulmonary Valves with Four Cusps	Bicuspid Aortic Valves	Aortic Valves with Four Cusps
Number classified as primary lesion	1	8	32	2
Number complicating other defects	30	4	46	2
Total	31	12	78	4

and transposition of the aorta. Additional anomalies were present in 56 (88 per cent) of 64 cases of bicuspid pulmonary valve collected from the literature by Dilg.[5] Patent interventricular septum occurred in all 56 cases, pulmonary stenosis in 26, transposition of the great vessels in 7 and patent ductus arteriosus in 6. There were no instances in which coarctation of the aorta was found. This is in contrast to congenital bicuspid aortic valve which is not infrequently accompanied by coarctation of the aorta.

In the present group of 9 bicuspid pulmonary valves, there were 2 that were isolated and 7 that were found with additional cardiac anomalies. The tetralogy of Fallot was present with 3 and a bicuspid aortic valve with 2, and with 1 of these there was also a widely patent foramen ovale. One was accompanied with a patent interventricular septum and another with transposition of the great vessels and cor biatriatum triloculare. The two hearts with bicuspid deformity of both the pulmonary and the aortic valves are distinctly unusual. Only 4 such hearts are recorded in the literature, according to deVries.[7] In general, an anomaly in the number of cusps of both semilunar valves of the heart

7. de Vries, W. M.: Beitr. z. path. Anat. u. z. allg. Path. **64**:39, 1918.

is rare. This is especially true of the combination of two cusps in one semilunar valve and four cusps in the other, only 2 cases of this type being mentioned in the literature so far as is known. In the first instance there occurred a bicuspid aortic valve and a pulmonary valve with four cusps,[7] while in the second there occurred a bicuspid pulmonary valve and an aortic valve with four cusps.[8] No record was found of a case with four cusps of both semilunar valves.

About one third of bicuspid pulmonary valves are associated with the tetralogy of Fallot. Conversely, however, hearts with the tetralogy of Fallot with few exceptions show a bicuspid pulmonary valve. Hence, although the tetralogy by common usage comprises a tetrad of abnormalities, i. e., pulmonary stenosis, patent interventricular septum, dextroposition of the aorta and hypertrophy of the right ventricle, there are usually five when the bicuspid pulmonary valve is added.

Morphologic Aspects.—Bicuspid pulmonary valves are all of congenital origin. As far as is known, no acquired deformity of this type has ever been observed. The bicuspid valves are of three types:

1. The simple bicuspid valve consisting of two cusps, which are normal in appearance and are usually of equal size.

2. The bicuspid valve in which one of the cusps is subdivided by a congenital ridge into two segments. There was only one in this study (case 7), and the ridge had the same distinctive morphologic appearance as in the congenital bicuspid aortic valve.[9] Grossly, it consisted of a narrow hemicylindric elevation of the pulmonary artery, extending from the level of the commissure to the bottom of the sinus of Valsalva; it projected only slightly into the sinus and was directed in the long axis of the pulmonary artery. Microscopically, the ridge consisted largely of elastica, whorled centrally and continuous laterally with that of the pulmonary media; at its termination the elastic tissue overlapped the annulus fibrosus both superficially and deeply.

3. The bicuspid valve in which one of the cusps reveals a small fibrous band, either longitudinal or wedge shaped, in the bottom of the sinus of Valsalva. This band may be thin and delicate or robust and prominent, is situated at or near the center of the cusp and projects only slightly into the sinus of Valsalva. It resembles a frenum connecting the pulmonary wall of the sinus with the base of the pulmonary cusp.

Although Dilg [5] and Babes [10] mentioned cases of congenital bicuspid pulmonary valve with a ridge or raphe in the sinus of Valsalva, a dis-

8. Volini, I. F., and Flaxman, N.: J. A. M. A. **111**:2001, 1938.

9. Koletsky, S.: Congenital Bicuspid Aortic Valves, Arch. Int. Med. **67**:129 (Jan.) 1941.

10. Babes, V.: Virchows Arch. f. path. Anat. **124**:562, 1891.

tinction between the congenital ridge and the fibrous band has not hitherto been made. The two deformities are readily differentiated on the basis of morphologic observations, as already described, and location. The different location in the sinus of Valsalva is shown in figure 1. The congenital ridge begins at the commissural level and terminates near the bottom of the sinus of Valsalva, and is thus situated along the upper pulmonary wall of the sinus. On the other hand, the fibrous band is situated in the bottom of the sinus of Valsalva, far below the commissural level, and it passes directly forward and obliquely from above downward across the attachment of the pulmonary cusp to insert into the latter's base.

Microscopically, while the congenital ridge is composed essentially of elastic tissue, which has a characteristic pattern and distribution,[9] the fibrous band contains no element of elastica. At its proximal attachment, either it lies superficial to the adjacent pulmonary media or the junction of fibrous and elastic tissue occurs on a more or less horizontal plane. This resembles the normal relationship of fibrous tissue to elastica at the commissures of semilunar valves, whereas in the congenital ridge, which represents a deformed commissure, the normal relationship is usually reversed and the terminal portion of the medial elastica overlaps the annulus fibrosus either superficially or both superficially and deeply.

Morphologically, the fibrous band resembles the subsidiary fibrous branch seen frequently at the terminal of a congenital ridge.[9] However, that it represents a commissural deformity from a genetic point of view is not clear.

No adequate data are available on the relative frequency of the three types of bicuspid pulmonary valve. My own experience indicates that the bicuspid valve with a true congenital ridge (type 2) is very rare, whereas that with a fibrous band in the sinus of Valsalva is comparatively more common, especially in hearts with the tetralogy of Fallot. The reverse is true of the bicuspid aortic valve. Of the 20 such valves in this survey, 9 were simple bicuspid valves, 10 revealed a conjoined cusp subdivided by a congenital ridge, and only 1 showed a fibrous band in the sinus of Valsalva. Moreover, in the last the fibrous band was so small that the valve might have been considered a simple bicuspid valve.

Embryologic Development.—Normally, the semilunar valves develop from four endocardial swellings in the distal portion of the truncus arteriosus. With the descent of the septum which partitions the common trunk into pulmonary artery and aorta, the two lateral swellings are bisected. As a result the aortic valve is derived from the original posterior and the posterior halves of the right and left swellings, while

the pulmonary valve is formed by the original anterior and the anterior halves of the lateral swellings. The swellings later become hollowed out to form the semilunar cusps.

De Vries [7] and Simonds [11] have discussed the possible mechanisms for the development of a congenital bicuspid semilunar valve. Theoretically, the deformity may have its genesis before or after the division of the common arterial trunk into pulmonary artery and aorta. In the former instance the anomaly is due either to (1) improper development of the endocardial swellings that form the rudimentary cusps, i. e., three swellings instead of four; (2) faulty growth or malposition of the septum which normally bisects the two lateral swellings, or (3) fusion of the two halves of the lateral swellings at the time of descent of the partitioning septum. This fusion may occur in one or both vessels. The development of a bicuspid valve after the division of the truncus arteriosus into pulmonary artery and aorta is due either to an abnormality of the hollowing out process of the endocardial pads of the truncus or to fusion of two cusps, or possibly to disappearance of one cusp. If, as some investigators claim, the third pulmonary and aortic cusps (anterior and posterior, respectively) arise after the separation of the truncus and the division of the original right and left endocardial swellings by the septum, failure of either of these cusps to develop would result in a bicuspid semilunar valve.

Congenital bicuspid pulmonary and aortic valves differ in several general respects; i. e., the pulmonary abnormality is much less frequently an isolated deformity of the heart than the aortic; the pulmonary anomaly is frequently accompanied by serious right-sided cardiac anomalies and the aortic by minor left-sided anomalies; a congenital ridge is rare in the bicuspid pulmonary valve and frequent in the bicuspid aortic valve. These differences indicate that the two deformities have a different origin. From the standpoint of genesis bicuspid semilunar valves due to a maldirected septum would be associated more frequently with other (and more serious) cardiac malformations than those due to fusion of two cusps after the separation of the pulmonary artery and aorta. The latter would be more likely to show a congenital ridge. Thus it appears probable that in many instances the bicuspid pulmonary valve arises prior to the division of the truncus arteriosus as the result of a maldirected partitioning septum, whereas the aortic anomaly develops after the division and is due to fusion of two of the semilunar cusps.

Neither the size of the two cusps nor their position appears to offer a reliable clue as to the exact genesis in any given case.

Clinical Significance.—In this study the congenital bicuspid pulmonary valves were found to be of little if any clinical significance. The

11. Simonds, J. P.: Am. J. M. Sc., **166**:584, 1923.

valves functioned competently in all instances, with no stenosis or regurgitation at the valvular orifice. Usually, and especially in the cases with the tetralogy of Fallot, the condition of the valve was merely incidental to far more serious cardiac deformities, such as pulmonary stenosis and transposition of the aorta. The two isolated bicuspid pulmonary valves were silent anomalies, discovered incidentally at autopsy.

None of the bicuspid pulmonary valves revealed superimposed inflammation or calcification. However, in 1923 Simonds [11] stated that, of 88 congenital bicuspid pulmonary valves collected from the literature, 21 showed endocarditis, an incidence of 24 per cent. How many of these presented true vegetative bacterial endocarditis is not known. Lack of susceptibility of the bicuspid pulmonary valve to superimposed endocarditis appears to be the general rule in cases of the tetralogy of Fallot, even though acute tricuspid valvulitis is not infrequently present. This is probably explained in part by the high intracardiac tension to which the tricuspid valve is subjected in these hearts, which the pulmonary valve escapes, owing to stenosis of the pulmonary conus.

SUMMARY

In 3,600 consecutive autopsies congenital bicuspid pulmonary valve was found in 8 (0.22 per cent). Three of these valves occurred in infants and children, and 5 in adults; 5 were in males and 3 in females. In the same group of autopsies were found 20 congenital bicuspid aortic valves, 6 pulmonary valves with four cusps and no aortic valves with four cusps. Two of the bicuspid pulmonary valves were isolated findings, while the remaining ones were associated with other cardiac anomalies. These were the tetralogy of Fallot, bicuspid aortic valve, patent interventricular septum, transposition of the great arterial trunks, cor biatriatum triloculare and widely patent foramen ovale.

Morphologically, there were 3 types of bicuspid pulmonary valve: (1) the simple bicuspid valve; (2) the bicuspid valve in which one of the cusps revealed a congenital ridge in the sinus of Valsalva; (3) the bicuspid valve in which one of the cusps revealed a small fibrous band or frenum in the sinus of Valsalva. The bicuspid pulmonary valve with a congenital ridge is apparently rare.

From the genetic point of view it appears probable that in many instances the formation of the bicuspid pulmonary valve is due to faulty growth or to malposition of the septum which partitions the common arterial trunk into pulmonary artery and aorta.

The bicuspid pulmonary valves in this series were of little if any clinical significance. They were silent, and there was no functional stenosis or insufficiency; the occurrence of such an anomaly was usually incidental to far more serious cardiac deformities. None of the valves showed superimposed inflammation or calcification.

CHORDAL ECTOPIA AND ITS POSSIBLE RELATION TO CHORDOMA

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The presence of aberrant collections of chordal tissue in the human embryo and fetus and the persistence of heterotopic vestiges of the human notochord in the cranial, vertebral and sacrococcygeal regions after birth have been previously demonstrated and recorded. It has been suggested that these aberrant chordal masses may be the starting point for neoplasia. This investigation has been undertaken in an effort to supplement the present knowledge of the variations in the persistence and regression of the human notochord, particularly the sacrococcygeal portion, and to discuss the possible relation of chordal ectopia in the occipital, vertebral and sacrococcygeal regions to chordoma.

REVIEW OF LITERATURE

Virchow [1] described jelly-like nodules appearing on the inner surface of the base of the skull as ecchordosis physaliphora, believing them to be cartilaginous growths. Müller [2] noted that portions of the notochord persist after birth in the base of the skull, in the odontoid process of the axis and in the coccyx, and he accurately ascribed these tumors to excessive growth of persistent notochordal tissue. Ribbert [3] produced similar tumors experimentally in rabbits by puncturing the intervertebral ligaments and allowing the escape of nucleus pulposus, and this led him to adopt the name "chordoma." These benign chordal ectopias in the cranial region (ecchordosis physaliphora spheno-occipitalis) are separated from chordoma, a true neoplasm of the notochord, although it has been suggested that these heterotopic vestiges may be the starting point for the development of neoplasia (Stewart and Morin[4]). Ecchordosis physaliphora spheno-occipitalis and malignant vertebral chordoma have been found in the same patient (Cappell [5]).

In the human embryo at the base of the skull the chorda dorsalis extends for a short distance on the posterior surface of the basilar cartilage, then travels forward and upward obliquely on the ventral surface, courses along this surface and extends backward to end in the region of the sella turcica. The middle

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From the Daniel Baugh Institute of Anatomy, Jefferson Medical College; Dr. J. Parsons Schaeffer, director.

1. Virchow, R.: Untersuchungen über die Entwicklung des Schädelgrundes, Berlin, G. Reimer, 1857; cited by Williams.[17]

2. Müller, H.: Ztschr. f. rat. Med. **2**:202, 1858; cited by Williams.[17]

3. Ribbert, H: Verhandl. d. Cong. f. inn. Med. **13**: 455, 1895.

4. Stewart, M. J., and Morin, J. E.: J. Path. & Bact. **29**:41, 1926.

5. Cappell, D. F.: J. Path. & Bact. **31**:797, 1928.

section bears a variable number of kinks, short branches and thickenings, and it may be attached at one or more points to the pharyngeal epithelium, which is often invaginated at these sites. These branches and irregularities of the cranial notochord may leave aberrant masses of chordal tissue lying free in the retropharyngeal connective tissue. From the work of Froriep,[6] Meyer,[7] Linck,[8] Huber,[9] Tourneux and Tourneux,[10] Snook[11] and others, it appears to be well established that the notochord plays an important part in the origin of the pharyngeal bursa.

Kunitomo,[12] in his investigations of the tail region of the human embryo, noted that prior to the 12 to 14 mm. stage the notochord passed through the vertebral column in a series of segmental undulatory curves, which tended to disappear, so that by the time the 18 mm. stage was present the main portion was straight, although the caudal portion became curved concomitantly. The human embryo was noted to possess a true tail, most completely developed in the 7.5 mm. stage and containing primitive vertebrae, a neural canal, a caudal gut and a notochord. This tail began to resorb in the 8 to 9 mm. embryo, after having added 10 to 12 caudal segments to the thirtieth segment between the fifth and sixth weeks. Regression occurred so rapidly that by the eighth week the coccygeal vertebrae were reduced to four or five segments. The caudal primitive vertebrae fused together, and the chorda within this fused segment became convoluted in the 25 to 30 mm. fetus. Later on, in the 37 mm. stage, chordal fragments became separated from the main chorda, which persisted below the thirtieth segment.

This process has been spoken of by Streeter[13] as retrogressive dedifferentiation, i. e., a reversion of tissue to an earlier embryonic type and subsequent redifferentiation into tissue of widely different histologic character. Any of the primitive structures in this region may leave a relic and contribute to one of the many histopathologic aberrations characteristic of the sacrococcygeal region (chordoma, neuroblastoma, teratoma, teratoid tumor, pilonidal and dermoid cysts, and tumors associated with spina bifida).

Linck and Warstat[14] reported 4 cases of small protrusions of chordal tissue on the anterior and posterior surfaces of the vertebral bodies in the lumbar region of the human fetus (40 to 50 mm.). They found throughout the vertebral bodies connecting branches between these foci and the main chordal strand, and they postulated that these ectopic collections of chordal tissue had been squeezed out of the bodies by the pressure of the developing cartilage. Bérard, Dunet and Peyron,[15] in a study of 50 human embryos and fetuses, found developmental displacements of chordal tissue in the sacrococcygeal region, lying beneath the perichondrium of the developing bones and in the surrounding mesenchyme on the anterior and posterior surfaces. These authors, also, attributed the displacements

6. Froriep, A.: Beiträge zur Anatomie und Embryologie, als Festgabe Jacob Henle, Bonn, 1882; cited by Huber.[9]
7. Meyer, R.: Anat. Anz. **37**:449, 1910.
8. Linck, A.: Ztschr. f. Ohrenh. **62**:158, 1911.
9. Huber, G. C.: Anat. Rec. **6**:373, 1912.
10. Tourneux, F., and Tourneux, J. P.: J. de l'anat. et physiol. **48**:57, 1912.
11. Snook, T.: Anat. Rec. **58**:303, 1934.
12. Kunitomo, K.: Contrib. Embryol. (no. 26) **8**:161, 1918.
13. Streeter, G. L.: Am. J. Anat. **25**:1, 1919.
14. Linck and Warstat: Beitr. z. klin. Chir. **127**:612, 1922.
15. Bérard, L.; Dunet, C., and Peyron, A.: Bull. Assoc. franç. p. l'étude du cancer **11**:28, 1922.

to extrusion by intravertebral growth pressure. Cappell [5] found in transverse sections of the lumbar region of a 21 mm. embryo small strands of chordal tissue extending from the central chordal mass into the surrounding mesenchyme and also small islets of notochordal cells on the anterior surface of the developing cartilage. Studnička [16] described such migrations of chordal cells from the main notochordal mass into the surrounding mesenchyme in transverse sections of a 10 mm. embryo and a 15 mm. embryo.

TABLE 1.—*Variations in Development Shown by Notochords of Normal Human Embryos and Fetuses*

Specimen	Crown-Rump Length, Mm.	Variations in the Notochord	Segment Level
1	4.0	None	..
2	5.5	None	..
3	6.6	None	..
4	7.5	None	..
5	8	None	..
6	11	None	..
7	12	None	..
8	12.6	None	..
9	15.5	One ventral bud	35
10	16	One dorsal bud; one dorsal bud	36, 37
11	17	None	..
12	17	None	..
13	18	One double ventral branch	34
14	19	One dorsal branch; tortuosity in coronal plane	34
15	20	None	..
16	21	One dorsal branch	34
17	23	Marked tortuosity in sagittal plane	..
18	23	One dorsal branch; marked tortuosity in sagittal plane	34
19	24	None	..
20	25	Three ventral buds; marked tortuosity in sagittal plane	Between 34 and 35
21	25	Three dorsal branches; separate chordal rests dorsal to main stem	33, 34, 35
22	26	None	..
23	16.4	None	..
24	27	None	..
25	27.5	None	..
26	30	One ventral branch; marked tortuosity in sagittal plane	34
27	33	None	..
28	33	One dorsal branch	33
29	35	None	..
30	36	None	..
31	37	One dorsal and two ventral buds	35
32	39	One ventral branch; one dorsal branch	33, 34
33	50	One ventral branch	32
34	50	None	..
35	52	One dorsal branch	33
36	67	None	..
37	70	None	..
38	70	None	..
39	75	None	..
40	88	None	..
41	115	Nests of chordal cells dorsal and ventral to main notochordal mass	4th segment from caudal extremity
42	118	Nests of chordal cells ventral to main notochordal mass	Terminal 3
43	128	None	..
44	132	None	..

MATERIALS AND METHODS

Fifty human embryos and fetuses were examined, which had been sectioned serially in the sagittal plane. Their crown-rump lengths varied from 4 to 132 mm. Normal specimens, nos. 1 to 36 (table 1), and abnormal specimens, nos. 45 to 50

16. Studnička, F. K.: Anat. Anz. **69**:520, 1930.

(table 2), were complete and were examined in the department of embryology of the Carnegie Institution of Washington, Baltimore, with the permission of Dr. George L. Streeter. The caudal ends of older fetuses, nos. 37 to 44 (table 1), were prepared at the Daniel Baugh Institute of Anatomy, Philadelphia, and were examined in collaboration with Dr. Andrew J. Ramsay. Graphic single plane reconstructions of the latter specimens were made by copying each section on transparent paper as thrown thereon from the projection apparatus and then superimposing all of the drawings on one sheet of paper.

OBSERVATIONS (TABLES 1 AND 2)

Many of the embryos and younger fetuses examined in this investigation had been used by Kunitomo [12] in his researches in the department of embryology of the Carnegie Institution of Washington,

TABLE 2.—*Development of the Notochord as Observed in Abnormal Human Embryos*

Specimen	Crown-Rump Length, Mm.	Developmental Abnormalities	Development of Notochord
45	9	Malformation of lower extremities	Normal
46	11	Malformation of lower extremities	Marked tortuosity in sagittal and coronal planes; dilatation of walls; termination within mass of undifferentiated mesenchyme; no budding, branching or ectopias
47	14	Spina bifida; anencephaly; maldeveloped central nervous system	Much coiling in coronal plane; no budding or branching
48	15	Malformation of lower extremities	Normal
49	16	Spina bifida; maldeveloped central nervous system	Much coiling in the coronal plane; no budding or branching
50	19	Spina bifida	Normal

and an opportunity was thereby afforded to confirm some of his observations on the regression of the tail end of the human notochord.

The number of vertebral segments increased to a maximum of 42 in the 11 mm. stage (specimen 6) and thereafter diminished by regression of the caudal end to an average number of 34 segments. Marked tortuosity and coiling of the notochord in the terminal segment appeared shortly after this process of regression commenced and was first evident in the 15.5 mm. specimen (9). This appearance suggested that the notochordal tissue of the regressed terminal segments had been crowded into the definitive terminal segment or segments. Such coiling was usually present in the sagittal plane (specimens 17, 18, 20 and 26), but in specimen 14 it was also present in the coronal plane (fig. 1 and table 1).

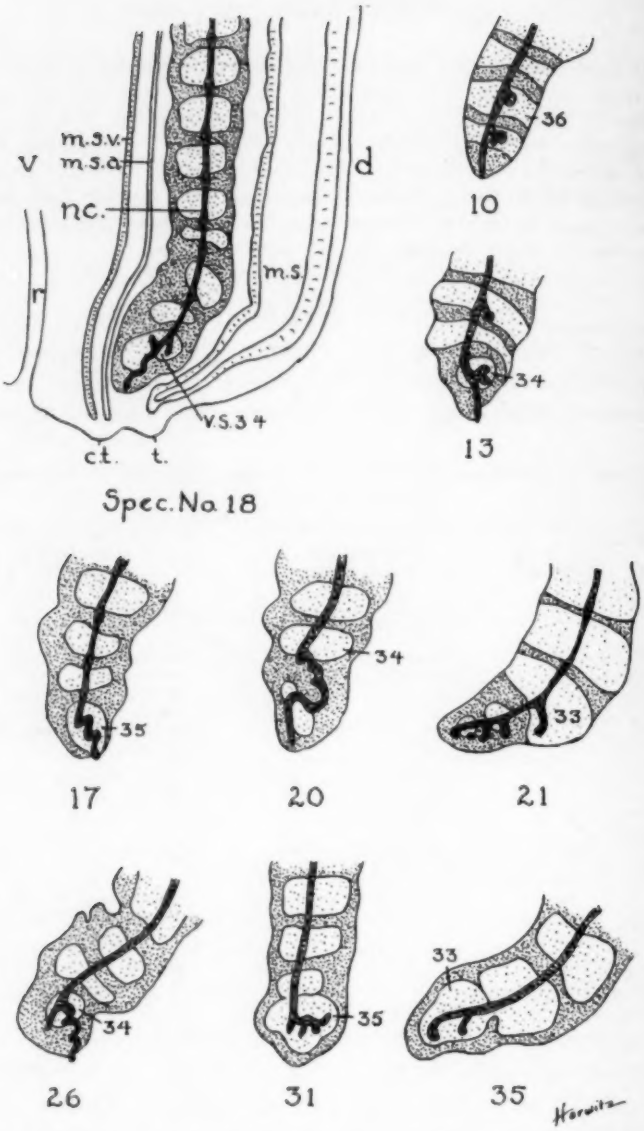

Fig. 1.—Variations in persistence and regression shown by sacrococcygeal notochords of normal human embryos and fetuses. The drawings represent sagittal reconstructions of the tail ends and illustrate ventral and dorsal budding and branching, and coiling, of the terminal notochord. In each specimen the ventral surface is on the left (*v*) and the dorsal surface is on the right (*d*) of the illustration; *nc.* indicates notochord; *v.s.*, vertebral segment; *m.s.*, medulla spinalis; *t*, rudiment of tail; *c.t.*, coccygeal tubercle; *r*, rectum; *m.s.a.*, medial sacral artery; *m.s.v.*, medial sacral vein. Specimen 18 represents an embryo at the stage of 23 mm.; specimen 10, 16 mm.; specimen 13, 18 mm.; specimen 17, 23 mm.; specimen 20, 25 mm.; specimen 21, 25 mm.; specimen 26, 30 mm.; specimen 31, 37 mm.; specimen 35, 52 mm.

One or more buds or branches of chordal tissue attached to the ventral or dorsal aspects of the main notochordal stem were present in 14 of the 44 normal specimens—ventral in 5, dorsal in 7 and both ventral and dorsal in 2; single in 9 and multiple in 5 (fig. 1 and table 1). Four of the 5 specimens which demonstrated marked coiling and irregularity of the caudal end of the notochord, already referred to, gave evidence of such offshoots from the main stem. The appearance in specimens 18, 20 and 26 suggested that such branching had developed consequent on coiling irregularities of the terminal chorda at this stage (fig. 1 and table 1).

A somewhat later stage was demonstrated in specimen 21 (crown-rump length, 25 mm.), where, in addition to the dorsal chordal branches, which were attached to the main notochordal mass, there were small nests of chordal tissue lying detached from and dorsal to the main stem (fig. 1, specimen 21). In specimens 41 (crown-rump length, 115 mm.) and 42 (crown-rump length, 118 mm.) chordal cells were found detached from and in the main ventral to the main notochordal mass. These nests of cells lay within the vertebral substance, reaching the ventral border in specimen 42 (fig. 2 *a* and *b*). Such chordal ectopias were not demonstrable in the older fetuses (specimens 43 and 44). They were not evident in embryos demonstrating skeletal maldevelopment (specimens 45 to 50) even though the notochord in 3 of these embryos had undergone considerable developmental alteration (table 2).

No notochordal ectopias were found in the cervical or thoracic regions of 36 complete human embryos and fetuses with crown-rump lengths between 4 and 67 mm. The cranial portion of the notochord was not investigated in this series.

COMMENT

Although the histopathology of chordoma, a tumor derived from notochordal tissue, has been clearly defined, there remains unexplained the predilection for occurrence in the cranial and sacrococcygeal regions rather than in the cervical, thoracic or lumbar portions of the vertebral column, where in the adult persistent chordal tissue is most abundant within the nucleus pulposus of the intervertebral disk. In addition, the growths diagnosed chordoma which have been located within the cervical, thoracic and lumbar portions of the spine have been noted to arise within vertebral bodies and not in intervertebral disks.

Three histologic stages of development are described in normal notochordal tissue (Williams [17]; Linck [18]). These stages in the ontogeny of the notochord may be reproduced with faithful exactitude in chordoma.

17. Williams, L. W.: Am. J. Anat. **8**:251, 1908.
18. Linck, A.: Anat. Hefte **42**:605, 1911.

The evidence indicates that there is a correlation between the degree of malignancy and the embryonic cell type comprising the neoplasm. In some cases the cells may be arranged as solid cords of undifferentiated cells; in other areas there may be vacuolation and the formation of actual physaliphorous cells, and extracellular mucin formation may be abundant; or the cells may be arranged in syncytial masses (Cappell [5]; Alézais and Peyron [19]). Mitotic figures, cellular pleomorphism and atypism, and tumor giant cells may be seen in the more malignant forms, but as a rule chordoma is well differentiated and of low malignancy,

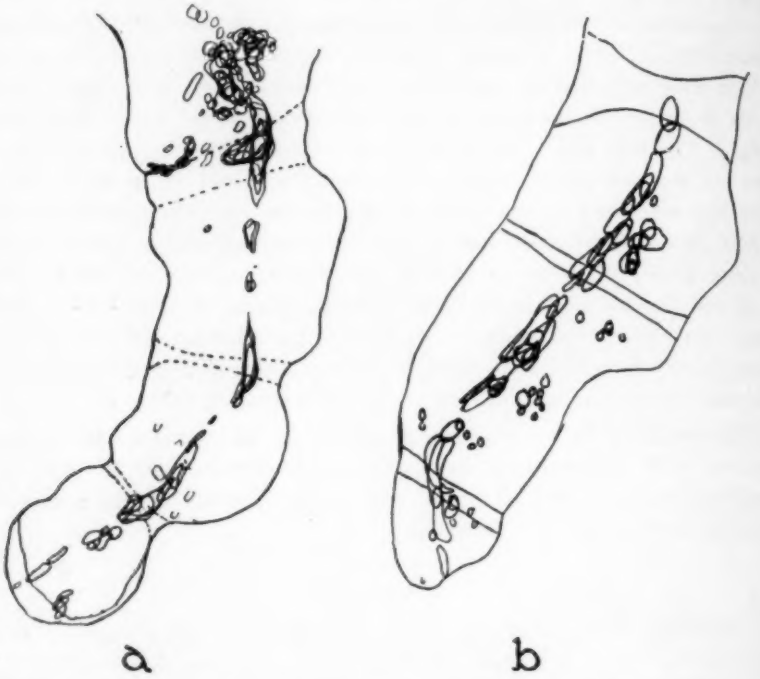

Fig. 2.—Variations in persistence and regression shown by sacrococcygeal notochords of normal human fetuses. In the drawing designated by *a* the ventral surface is on the left, and in that designated by *b* it is on the right. (*a*) Sagittal reconstruction of the tail end of fetus 41 (115 mm.). Detached nests of chordal tissue lie ventral and dorsal to the notochord within the fourth vertebral segment from the caudal extremity. (*b*) Sagittal reconstruction of the tail end of fetus 42 (118 mm.). Detached nests of chordal tissue lie ventral to the notochord within the terminal three vertebral segments.

with a tendency to be locally invasive and destructive, and to metastasize only when sacrococcygeal (Mabrey [20]).

19. Alézais and Peyron: Compt. rend. Soc. de biol. **83**:368, 1920.
20. Mabrey, R. E.: Am. J. Cancer **25**:501, 1935.

From a review of the literature and the confirmation of previous reports by this investigation, it is evident that chordal displacements from the sacrococcygeal portion of the notochord occur frequently in the normal development and regression of the human notochord. Tortuosity and coiling of the caudal end of the notochord appear early in the terminal segment or segments, following regression of the tail end, and this is followed by ventral and dorsal (and probably lateral) budding and branching from the main chordal stem. These chordal offshoots become separated from the main stem and are driven to the periphery by the same intravertebral growth tension which serves to compress the intravertebral portion of the notochord and to express the latter's contents into the intervertebral areas. Nests of chordal cells which are driven to the periphery might conceivably survive. Although chordal ectopias were not demonstrated in the sacrococcygeal region of 2 fetuses past the 118 mm. stage in this series, they might have been revealed in some of the specimens of a larger group of fetuses beyond this stage, since aberrant chordal masses have been demonstrated *after birth* in the cranial region, vertebral bodies and sacrum.

Frequently, benign chordal masses have been found on the inner surface of the base of the adult skull (ecchordosis physaliphora). Schmorl (cited by Beadle [21]) demonstrated the occurrence of chordal remnants *within* the vertebral bodies in 8 adult human cadavers (thoracic vertebrae in 7, lumbar vertebra in 1) in his examination of more than 5,000 human spinal columns removed at autopsy. In 3 specimens the chordal remains were centrally located, in 4 specimens the remnants were posteriorly placed, and in 1 instance they were located laterally. Nests of well preserved chordal cells were demonstrated by Schwabe [22] within the disk zones of the sacrum in every decade of life up to the fifth decade, and, in a degenerated stage, even thereafter. These strands of cells were observed to extend to the surfaces of the sacrum and to the margins of the spinal canal. Since the sacral segments, like the coccygeal segments, become fused together, the notochordal remnants within the fibrocartilaginous disks of the sacrum and coccyx are intraosseous, a position resembling that of the chordal vestiges within the vertebral bodies of the specimens described by Schmorl.

The demonstration of aberrant chordal masses in the cervical, thoracic, lumbar and sacrococcygeal regions of the spinal column, as well as in the cranial region, suggests that these heterotopic vestiges may be the starting point for neoplasia. The anatomic distribution in 245 cases of chordoma collected from the literature in shown in table 3. This topographic distribution supports the view that these tumors arise

21. Beadle, O. A.: Medical Uses of Radium, Medical Research Council, Special Report Series, no. 160, London, His Majesty's Stationery Office, 1931, p. 5.

22. Schwabe, R.: Virchows Arch. f. path. Anat. **287**:651, 1933.

from heterotopic chordal vestiges rather than from those within the nucleus pulposus of the intervertebral disk. It is noteworthy that the vertebral chordoma has invariably arisen within the vertebral body and not in the intervertebral disk. The concept of an origin from the chordal remnants within the nucleus pulposus of the intervertebral fibrocartilages would not explain the occurrence of chordal masses in the cranial region.

Additional factors must be sought to explain (1) why metastases have not been demonstrated in autopsies in cases of malignant sphenoccipital chordoma but have been found in a relatively high percentage (62.5) of reported autopsies in cases of the malignant sacrococcygeal type (Mabrey [20]) and (2) why chordoma rarely manifests itself in infancy and occurs infrequently prior to the third decade of life (Montgomery and Wolman [23]; Mabrey [20]). The only true congenital malignant chordoma to be reported was that described by Hennig [24] in the sacrococcygeal region of a 7 month stillborn boy. Chordoma has been demonstrated shortly after birth, however—in the oral region by

TABLE 3.—*Distribution of Chordoma as Reported in the Literature*

Region		Cases	Percental Incidence
Cranial		81	33.1
Vertebral		29	11.8
Cervical	11		
Thoracic	6		
Lumbar	12		
Sacrococcygeal		135	55.1
Total		245	100.0

Koritsky [25] and Rubaschow [26] and in the sacrococcygeal region by Montgomery and Wolman.[23]

SUMMARY

The results of this investigation serve to corroborate and supplement the present knowledge of the variations in the regression of the human notochord and the persistence of aberrant chordal tissue in the human embryo and fetus and in the cranial, vertebral and sacrococcygeal regions after birth. Such heterotopic chordal vestiges may be the starting point for neoplasia. Their topographic distribution corresponds closely to the sites of occurrence of chordoma, and this is interpreted as supporting the view that chordoma arises from the aberrant chordal vestiges rather than from the chordal remnants within the nucleus pulposus of the intervertebral disk.

23. Montgomery, H., and Wolman, I. J.: Am. J. Dis. Child. **46**:1263, 1933.
24. Hennig, L.: Beitr. z. path. Anat. u. z. allg. Path. **23**:593, 1900.
25. Koritsky, G. E.: Kharkov M. J. **17**:62, 1914; abstracted, Centralbl. f. allg. Path. u. path. Anat. **25**:638, 1914.
26. Rubaschow, S.: Centralbl. f. Chir. **56**:137, 1929.

VALUE OF MALLORY'S ANILINE BLUE STAIN IN THE HISTOPHYSIOLOGIC DIAGNOSIS OF GOITER

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There is still disagreement about whether toxic goiter is caused by excessive secretion of normal thyroxine or by a chemical alteration of this hormone.

The assumption of Plummer [1] that in exophthalmic goiter the thyroxine molecule is incompletely iodized lacks support by chemical analysis.

Since colloid is generally regarded as the physical substratum of the active secretion of the thyroid, the question arises whether in cases of toxic goiter a chemical alteration of the colloid can be demonstrated by staining reactions.

In 1923 and 1927 Troell [2] reported on the use of Mallory's stain in the study of 161 specimens of goiter. He contended that this method allows one to differentiate between toxic and nontoxic goiter, regardless of cellular changes. According to Troell, the colloid in exophthalmic goiter stains blue with Mallory's stain and that in nontoxic goiter and the normal thyroid stains red. While in his cases of diffuse goiter the differential stain conformed with the clinical symptoms, Troell admitted that in cases of nodular goiter the relationship was less definite. However, the blue color at least predominated in toxic nodular goiters.

The results of Troell's studies were not confirmed by Hueck [3] and Neuweiler.[4] Hueck found in 1 exophthalmic goiter only red-stained colloid; in 2, half red-stained, half blue-stained colloid. Neuweiler found blue colloid not only in the goiters of cretins, accompanied with symptoms of decreased thyroid function, but also in nontoxic goiters. Often both colors were present in the same thyroid and even in the same follicle.

From the Department of Pathology, St. Francis Hospital.

1. Plummer, H. S.: The Thyroid Gland, St. Louis, C. V. Mosby Company, 1926.

2. Troell, A.: Arch. f. klin. Chir. **124**:731, 1923; **146**:754, 1927.

3. Hueck, H.: Arch. f. klin. Chir. **130**:178, 1924.

4. Neuweiler, W.: Zentralbl. f. allg. Path. u. path. Anat. **36**:145, 1925.

While Hueck and Neuweiler denied any value of Mallory's stain in the diagnosis of goiter, Mayer and Fürstenheim [5] felt that this stain gave better information on the activity of the thyroid than a study of the epithelial changes. These authors were, however, not of Troell's opinion that the difference in the staining reaction of the colloid is caused by chemical alteration of the secretion, but believed it was due to a difference in concentration.

In this country, no clinical-pathologic studies of goiter using Mallory's stain have been reported. Only Hertzler,[6] in a recent paper on the degenerative goiter heart, recommended a modification of Mallory's stain as a specific indicator of cardiotoxic colloid.

MATERIAL AND METHODS

This paper is based on the clinical-pathologic study of 50 cases of goiter. As controls, about the same number of normal thyroids from human autopsy material and from animals were stained.

The thyroids were fixed in Zenker's solution, embedded in paraffin and sectioned 6 to 8 microns thick. After removal of the paraffin by means of xylene, the sections were stained with Heidenhain's modification of Mallory's aniline blue collagen stain, the directions for which are as follows:

1. Treat with 5 per cent aqueous solution of acid fuchsin for five minutes.
2. Wash quickly in distilled water.
3. Treat with 5 per cent phosphotungstic acid for fifteen to thirty minutes.
4. Wash quickly in distilled water.
5. Stain for fifteen minutes in Heidenhain's aniline blue, which is made as follows:

Aniline blue	0.5	Gm.
Orange G	2	Gm.
Distilled water	100	cc.
Acetic acid, glacial	8	Gm.

Boil and filter after cooling. For staining, dilute this stock solution 1:3 with distilled water.

6. Wash quickly in distilled water.
7. Differentiate in 95 per cent alcohol for five to ten minutes. Nuclei become red; collagen, blue.
8. Clear with absolute alcohol, and then xylene. Mount in balsam.

RESULTS

Table 1 summarizes the clinical-pathologic findings. The clinical grade of toxicity was expressed in the following way:

\+ mild hyperthyroidism
\+ + severe hyperthyroidism without ocular signs
\+ + + complete clinical picture of exophthalmic goiter

5. Mayer, E., and Fürstenheim, A. C.: Virchows Arch. f. path. Anat. **278**:391, 1930.

6. Hertzler, A. E.: Arch. Surg. **38**:417, 1939.

The histologic diagnosis of each goiter was made from paraffin sections stained with hematoxylin and eosin. Aschoff's classification [7] was used. In the last column of table 1 the staining reaction of the colloid with

TABLE 1.—*Summary of Results of Histophysiologic Study of Fifty Cases of Goiter*

Case	Sex	Age	Clinical Diagnosis	Histologic Diagnosis	Mallory's Stain	
					Blue	Red
1	M	40	+++	Exophthalmic goiter	90	10
2	F	27	+++	Exophthalmic goiter	20	80
3	F	43	+++	Nodular colloid goiter with epithelial proliferation	10	90
4	F	39	+++	Exophthalmic goiter	100	0
5	F	29	+++	Exophthalmic goiter	90	10
6	F	26	+++	Exophthalmic goiter	90	10
7	F	26	+++	Exophthalmic goiter	80	20
8	F	24	+++	Exophthalmic goiter	95	5
9	F	28	+++	Exophthalmic goiter	80	20
10	F	39	+++	Exophthalmic goiter	90	10
11	F	37	+++	Exophthalmic goiter	80	20
12	F	31	+++	Exophthalmic goiter	98	2
13	F	35	+++	Exophthalmic goiter	100	0
14	F	46	+++	Exophthalmic goiter	90	10
15	F	..	+++	Diffuse and nodular colloid goiter with epithelial proliferation	100	0
16	F	23	++	Diffuse colloid goiter with epithelial proliferation	80	20
17	F	52	++	Exophthalmic goiter	100	0
18	F	55	++	Exophthalmic goiter	Unstained colloid	
19	F	32	++	Exophthalmic goiter	80	20
20	F	41	++	Diffuse colloid goiter	10	90
21	F	56	++	Nodular hyperplasia	0	100
22	F	33	++	Diffuse and nodular colloid goiter with epithelial proliferation	75	25
23	F	20	++	Colloid adenoma	10	90
24	F	35	++	Diffuse colloid goiter	80	20
25	F	52	++	Colloid adenoma	40	60
27	F	58	+	Diffuse colloid goiter	10	90
28	F	23	+	Diffuse colloid goiter	10	90
29	F	47	+	Colloid adenoma	10	90
30	F	29	+	Diffuse and nodular colloid goiter	10	90
31	F	44	+	Diffuse and nodular colloid goiter with epithelial proliferation	20	80
32	F	61	+	Nodular colloid goiter with epithelial proliferation	10	90
33	F	30	+	Diffuse colloid goiter	40	60
34	F	32	+	Diffuse and nodular colloid goiter	10	90
35	M	28	+	Diffuse colloid goiter	10	90
36	F	54	+	Nodular colloid goiter	20	80
37	F	62	+	Colloid adenoma	90	10
38	M	45	+	Lymphosarcoma	10	10
39	F	24	+	Diffuse colloid goiter	90	10
40	F	42	0	Fetal adenoma	90	10
41	M	20	0	Fetal adenoma	90	10
42	F	46	0	Fetal adenoma	60	40
43	F	15	0	Colloid adenoma with papillary proliferation	80	20
44	M	..	0	Single colloid cyst	0	100
45	F	27	0	Lymphadenoid goiter	100	0
46	F	53	0	Nodular colloid goiter and fetal adenoma	20	80
47	F	35	0	Fetal adenoma	20	80
48	F	..	0	Fetal adenoma	100	0
49	F	40	0	Lymphadenoid goiter	100	0
50	F	75	0	Nodular colloid goiter with epithelial proliferation	80	20

+, mild hyperthyroidism; ++, severe hyperthyroidism without ocular signs; +++, complete clinical picture of exophthalmic goiter.

Mallory's stain is given on a percentage basis. Estimations of the staining reaction were made without previous knowledge of the clinical diagnoses.

7. Hellwig, C. A.: Arch. Path. **28**:870, 1939.

Fifteen of the cases studied were cases in which all the symptoms of exophthalmic goiter were presented. All specimens were diagnosed by the usual histologic methods as exophthalmic goiter except 1, which was a nodular colloid goiter with epithelial proliferation. With Mallory's stain, the following results were obtained in this group. In 3 specimens the colloid was stained exclusively blue. In 10 specimens 80 per cent or more of the acini were filled with blue-stained colloid. The red colloid predominated in only 2 specimens. Of these, one was from a woman who had been treated for six months with compound solution of iodine U. S. P. The other was from a patient whose condition had been diagnosed clinically as exophthalmic goiter but who had a nodular colloid goiter with hyperplasia, in which only 10 per cent of the colloid was stained blue.

In the second group of 10 cases, representing severe hyperthyroidism without exophthalmos, 6 thyroids contained more blue than red colloid. Five of these specimens were diffuse goiters. One was a diffuse and nodular colloid goiter. Of the remaining 4, 2 were nodular colloid goiters with more red than blue colloid. A 56 year old woman with a basal metabolic rate 72 per cent above normal, who had died after operation in a thyrotoxic crisis, had nodular hyperplasia with only red-stained colloid. A 55 year old woman who died of heart failure without operation had histologically typical exophthalmic goiter with completely unstained colloid.

There were 13 cases of mild hyperthyroidism in my series. Most of the goiter specimens were classified as diffuse or nodular colloid goiters. It is conceded that the clinical and histologic diagnosis of mild hyperthyroidism is most difficult. Therefore, a differential stain for toxic colloid would be of great value especially in this group of cases. However, the results of Mallory's stain were disappointing. Only 4 of the 13 specimens had more than 80 per cent blue-stained colloid. One of those was a lymphosarcoma of the thyroid in a 45 year old man who had definite symptoms of hyperthyroidism.

The last group of my series consisted of 11 cases of nontoxic goiter. There were 5 specimens of fetal adenoma, 4 of nodular colloid goiter and 2 of lymphadenoid goiter. The colloid stained predominantly red in only 4 of the 11 specimens. In the 2 specimens of adenoid goiter all the acini contained blue-stained colloid in spite of the fact that the patients did not show any toxic symptoms. All the specimens of fetal adenoma except 1 had much more blue than red colloid.

In table 2 the material is arranged according to the classification of the American Society for the Study of Goiter. Of the 26 thyroids showing diffuse toxic goiter, 19 had a high percentage of blue-stained colloid.

Of the 11 nodular toxic goiters, only 2 had more blue than red colloid, and of the 9 nontoxic nodular goiters, 6 had predominantly blue-stained colloid.

CORRELATION OF CLINICAL AND PATHOLOGIC OBSERVATIONS

From the foregoing study of 50 cases of goiter it seems evident that the value of Mallory's stain in the histophysiologic analysis of goiters is limited. My results do not support the contention of Troell that blue colloid represents a chemical alteration of the thyroid hormone which is specific for toxic goiter. While it is true that exophthalmic goiters have, as a rule, blue-stained colloid, one has to consider first that in most exophthalmic goiters red-stained colloid is also present and second that many nontoxic goiters, especially those classified as fetal adenoma, have predominantly blue-stained colloid.

From the standpoint of the clinical pathologist, the addition of Mallory's stain to the routine methods of histologic studies of goiter

TABLE 2.—*Staining of Colloid in Various Types of Goiter When Mallory's Aniline Blue Stain Was Used*

Types of Goiter	Blue Colloid Predominating	Red Colloid Predominating
Diffuse nontoxic goiters	2	0
Diffuse toxic goiters	19	7
Nodular nontoxic goiters	6	3
Nodular toxic goiters	2	9

seems unnecessary, because the only form of goiter with which Mallory's stain gives uniform results is exophthalmic goiter. Here the cellular changes are as a rule so characteristic that a diagnosis can be made from routine sections stained with hematoxylin and eosin. In the group of toxic nodular goiter, in which cellular changes are often absent, a stain which would differentiate between toxic and nontoxic colloid would be of greatest value. From the present study, however, it is apparent that Mallory's stain does not fulfil the requirements of such a differential stain.

As to the question of whether chemical or physical properties of the colloid are responsible for the different color with Mallory's stain, the view held by Hewer [8] that fresh, thin colloid is more alkaline and combines with aniline blue, while stored, thick colloid is more acid and has an affinity to fuchsin seems plausible. The staining variations would depend, therefore, on the acid-alkaline reaction of the colloid. In my control series of human and animal thyroids, the colloid of fetal glands stained exclusively blue. In favor of the view that the hydrogen ion con-

8. Hewer, E. J.: J. Path. & Bact. **30**:621, 1927.

centration of the colloid determines the color of the colloid is the common occurrence of particolored reactions in the same acinus. There is often a central mass of colloid giving a red color with a peripheral blue rim. Whether this blue colloid is in a state of resorption or whether it is freshly secreted cannot be decided by the study of fixed stained material.

SUMMARY

It appears that Mallory's aniline blue stain is not a differential stain for toxic colloid. The blue color indicates not a specific chemical alteration of the colloid but only a more liquid and more acid state. It is true that Mallory's stain gives uniform results in exophthalmic goiter. But in this form of goiter the cellular changes are as a rule characteristic enough to allow one to make a diagnosis by the usual histologic methods.

Mallory's stain does not help in the histophysiologic study of nodular toxic goiter, in which epithelial proliferation is often absent and the cellular changes seldom allow one to make a functional diagnosis. Toxic nodular goiter is still a clinical, not an anatomic, diagnosis.

The colloid in normal thyroids from fetuses and infants was stained almost exclusively blue.

AN ANATOMIC BASIS FOR AURICULAR FIBRILLATION

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In an attempt to demonstrate a cardiac lesion which is associated with auricular fibrillation, I studied the musculature of two rheumatic hearts with mitral stenosis, for both of which there were electrocardiographic records of auricular fibrillation. These muscle tissues were compared with those of the auricles of other hearts, some with and some without disease. In this connection it is to be recalled that the endocardium of the left auricle is normally supplied with smooth muscle fibers.[1]

Sections of tissues embedded in celloidin (a preparation of pyroxylin) were used because the microscopic pictures are less distorted and with hematoxylin and eosin there is better differentiation between smooth muscle, cardiac muscle and connective tissue.

Variable numbers of sections were taken at random from each left auricle. Measurements were made with a centimeter scale, a micrometer and by counting cells along a straight line when an intermixture of muscle and connective tissue made accurate measurements of groups of muscle cells impracticable.

The first case was that of a 33 year old white woman whose first episode of rheumatic fever had occurred seventeen years before, and whose last occurred four years before admission to the hospital. Between these two she had had six others of like nature. During the last four years she had constant dyspnea and orthopnea. She had received digitalis during the preceding year. On admission there was auricular fibrillation which continued until death, about one month later. At necropsy there was moderate mitral stenosis, and the left auricle was enormously dilated. Microscopically, the endocardial smooth muscle of the left auricle was abundant and was arranged as transverse and longitudinal coats. The cardiac muscle (myocardium) consisted of scattered groups of muscle fibers which showed large, deeply staining homogeneous irregular nuclei of angular shape. Some nuclei seemed to

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From the Laboratories of Pathology, Bellevue Hospital.

1. Maximow, A. A., and Bloom, W.: A Textbook of Histology, ed. 3, Philadelphia, W. B. Saunders Company, 1938, p. 247.

have no cytoplasm around them. A few lymphocytes were scattered among the muscle fibers.

The second case was that of a 28 year old white man who was told eight years before admission that he had heart disease. He had experienced frequent hemoptysis and dyspnea. On admission he had auricular fibrillation which continued until death, two and one-half months later. He had received digitalis for five weeks. At necropsy there was marked mitral stenosis. The left auricle was dilated, and its wall was of irregular thickness. Microscopically, it showed smooth muscle which was frequently arranged in transverse and longitudinal layers. In some places this muscle was thick, and in others it was absent. Lymphocytes occurred in small scattered clumps but were sometimes numerous. The cardiac muscle bundles varied up to 4 mm. in thickness. In some parts, both smooth and cardiac muscle were absent. The nuclei of the

TABLE 1.—*Comparison of Cases by Weights and Measurements*

Case	Age	Heart Weight, Gm.	Endocardial Smooth Muscle, Mm.	Endocardial Thickness, Mm.	Myocardial Thickness, Mm.	Total Auricle Thickness, Mm.
1	52	564	0.055	0.67	2.4	3.5
2	64	441	0.042	0.76	1.85	2.8
3	51	406	0.058	0.63	1.9	3.1
4	9 mo.	...	0.031	0.40	1.5	2.6
5	18 days	...	0.0	0.40	1.4	2.3
6	15	...	0.033	0.54	1.3	2.0
7*	33	570	0.530	0.86	0.2	1.8
8*	28	630	0.171	0.60	2.0	3.1
9*	42	500	0.125	0.46	2.3	3.0
10*	49	370	0.113	0.54	1.0	1.9

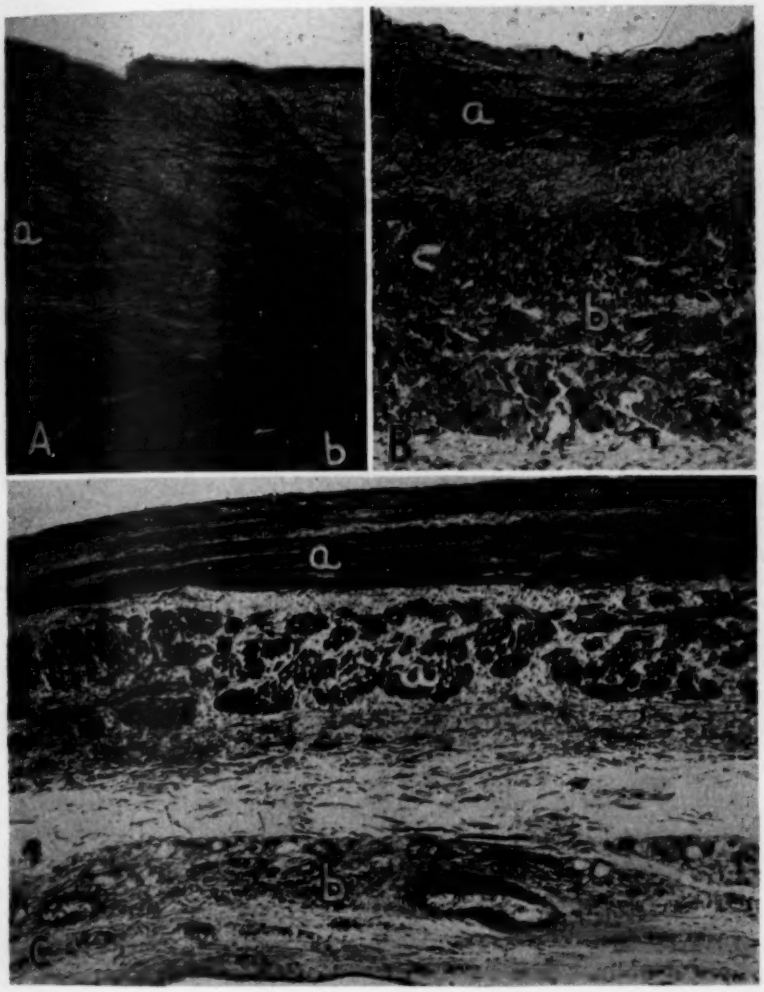
* Case 7 is the first case described in this paper, and case 8 is the second. Case 9 is an instance of mitral stenosis without auricular fibrillation; case 10, vegetative endocarditis.

cardiac muscle were similar to those described in the first case. The cytoplasm showed great variations. Many fibers were well preserved. This was particularly true in the thicker areas, while the fibers in the thinner areas were clear, shadowy and devoid of myofibrils.

The controls were 1 patient with mitral stenosis without a history of rheumatic fever or of auricular fibrillation; 1 patient with subacute bacterial endocarditis without a history of rheumatic fever but with slight thickening of the chordae tendineae; 6 patients with hypertensive and arteriosclerotic heart disease, death following cardiac decompensation; 4 patients with arteriosclerotic heart disease and with other lesions, which were the cause of death; 1 patient with patent foramen ovale; 1 patient with hydrocephalus; 4 patients with carcinoma; 4 with lobar pneumonia; 1 patient with pneumococcic meningitis and endocarditis; 1 patient with bronchiectasis and abscess formation; 1 with acute peritonitis; 1 patient with pulmonary abscess, and 1 infant.

In table 1 the cases of these patients are tabulated as those of: patients dying of arteriosclerotic heart disease (designated as 1 in the

table); patients dying of other causes with accompanying arteriosclerotic heart disease (2); patients dying of other diseases (3); children (4, 5 and 6); patients with disease of the mitral valve (7, 8, 9 and 10). With the exception of children and patients with disease of the mitral



A, normal auricle: (*a*) occasional endocardial smooth muscle cells; (*b*) myocardium.

B, auricle from a man with auricular fibrillation: (*a*) endocardial smooth muscle is prominent; (*b*) the myocardium is degenerate.

C, auricle from a woman with auricular fibrillation: (*a*) endocardial smooth muscle arranged longitudinally and transversely; (*b*) myocardium absent except for its connective tissue remains.

valve, each of these being considered separately, an average of each group is made.

If the auricles are considered as rectangular bodies, the total volumes of the various parts may be determined by multiplying the surface areas by the thicknesses of the different layers (table 2).

COMMENT

In the first case, the total volume of smooth muscle was about sixty times greater than that in the control, while the cardiac muscle was only about one half that of the control. The cardiac muscle fibers were so badly degenerated and so widely separated that it seemed doubtful that they were capable of affecting the auricle at all. In the second case, the volume of smooth muscle was about five times that of the average, while that of the cardiac muscle was slightly greater. In the auricles of the patients with disease of the mitral valve and no evidence of auricular fibrillation, the cardiac muscle was well preserved, although there was definite increase of smooth muscle. Since in auricular dilatation smooth muscle seems to hypertrophy and cardiac muscle, after first hypertrophying, atrophies, a state eventually arrives in which the cardiac muscle is incapable of further work and the hyperplastic smooth muscle substitutes for it. In these circumstances the behavior of a fibrillating auricle would resemble, for example, that of an overdistended urinary bladder.

TABLE 2.—*Comparison of Cases by Computations*

	Total Auricular Volume, Cc.	Endocardial Smooth Muscle, Cc.	Endocardial Volume, Cc.	Cardiac Muscle, Cc.	Auricular Surface, Sq. Cm.
First case	67.1	19.7	32.0	7.4	373.1
Second case	28.6	1.5	5.5	18.4	92.3
Mitral stenosis without auricular fibrillation	21.6	0.9	3.3	16.5	72.0
Cardiac arteriosclerosis	21.0	0.3	4.0	14.4	60.0

SUMMARY

It is believed that auricular fibrillation in patients with chronic rheumatic heart disease may be due to substitution of auricular smooth muscle for striated cardiac muscle.

CARDIAC HYPERTROPHY AND CORONARY ARTERIOSCLEROSIS IN HYPERTENSION

JOSEPH R. KAHN, M.D.
AND
EDGAR S. INGRAHAM JR., M.D.
CLEVELAND

In prolonged hypertension in man, the weight of the hypertrophic heart is not always closely correlated with the degree and duration of the hypertension. In persistent experimental hypertension in the dog, it has not been found possible to demonstrate anything more than a tendency toward cardiac hypertrophy. Such observations as these indicate that some factors other than the elevation of blood pressure exercise an influence in the development of the hypertrophy. One of the variables is the condition of the coronary arteries, which in the dog are not demonstrably diseased and in man are usually the seat of arteriosclerosis of different degrees.

On the basis of clinical histories and autopsy protocols, a statistical analysis has been made of 1,000 cases of prolonged hypertension in an attempt to determine whether or not coronary arteriosclerosis plays a part in the development of cardiac hypertrophy. Only those cases in which the systolic and diastolic pressures were 160 and 90 mm. of mercury or more, respectively, were included in the study. The state of the general nutrition could not always be determined with precision and was not considered in the selection of cases. Those in which on critical examination of the clinical and autopsy records the diagnosis of hypertension was found to be doubtful and those in which there was an anatomic diagnosis of disease of the cardiac valves were excluded from the series. The records were collected from the University Hospitals of Cleveland and the City Hospital, Cleveland, and, through the aid of Drs. S. E. Dorst and R. S. Austin, from the Cincinnati General Hospital.

The cases were divided into group 1, with slight or no coronary arteriosclerosis, and group 2, with moderate to marked coronary disease; this group was redivided into groups 3 and 4, with moderate and marked coronary arteriosclerosis, respectively. All these groups were subdivided as to sex and then redivided according to age, in ten year periods.

From the Institute of Pathology, Western Reserve University, and the University Hospitals of Cleveland.

Statistically adequate groups were obtained in both sexes in the periods between 35 and 85 years. For each age group the arithmetic mean and the standard deviation of the mean weight of the hearts were calculated. The difference between the mean weights of the hearts of the two groups was found, and the standard error of the differences between the means calculated. A difference between the means greater than twice the standard error was considered statistically significant.

The material is tabulated so as to compare the differences between the cardiac weights of (*a*) hypertensive patients with slight or no coronary sclerosis and those with moderate and marked sclerosis, (*b*) those with slight or no sclerosis and those with marked sclerosis and (*c*) those with slight or no sclerosis and those with moderate sclerosis. The first of these comparisons is set up in tables 1 and 2. Table 1, dealing with males, shows that except for the group aged 25 to 34 years and that aged 75 to 84 years there is in all age groups a significantly greater cardiac weight in those with moderate and marked coronary sclerosis than in those with slight or no coronary sclerosis. Table 2, dealing with females, shows a statistically adequate difference in those three groups aged from 35 to 64 years.

The second comparison is made in tables 3 and 4. Table 3, dealing with males, shows that in the age groups from 35 to 74 years those with marked coronary sclerosis have significantly greater cardiac weights than those with slight or no sclerosis. In the group aged 75 to 84 years the situation is reversed. Table 4, dealing with females, shows that the differences are statistically valid in all between 35 and 84 years of age except for the group between 65 and 74 years of age.

The third comparison is made in tables 5 and 6. In table 5, dealing with males, those with moderate coronary sclerosis have significantly greater cardiac weights than those with slight or no sclerosis in the age periods between 35 and 74 years. Table 6, dealing with females, shows a statistically valid difference in only one group, that of 45 to 54 years.

The mean weight of the hearts of those with marked coronary sclerosis is slightly greater than that of the group with moderate and marked sclerosis, and both these mean weights are slightly greater than that of the patients with moderate coronary sclerosis. The differences in the mean weights of those with marked sclerosis and those with marked and moderate sclerosis as compared with those with slight or no sclerosis are fairly large. In all these instances the factor of error is so great that the differences cannot be considered to be more than trends.

If it is admitted that the differences in certain age groups are trends, the departures are in the higher ages. The age groups of from 35 to 64 years show significant differences in cardiac weights. It is at this time of life that hypertension is of greatest clinical importance and is likely

to be most marked. In this period, also, nutritional disturbances and incidental diseases are less likely to occur than in advanced life. Thus, in spite of discrepancies in the figures for the more aged, it is at least

TABLE 1.—*Comparative Weights of Hearts of Hypertensive Males with Moderate and Marked Coronary Arteriosclerosis and of Those with Slight or No Coronary Arteriosclerosis*

Age Group	Moderate and Marked Coronary Arteriosclerosis: Cases	Moderate and Marked Coronary Arteriosclerosis: Mean Heart Weight with Standard Deviation	Slight or No Coronary Arteriosclerosis: Cases	Slight or No Coronary Arteriosclerosis: Mean Heart Weight with Standard Deviation	Difference Between Means	Standard Error of Difference Between Means
15-24	0	0	1	375	.....	....
25-34	7	581 ± 104	14	493 ± 94	+ 88	±47
35-44	38	626 ± 123	32	491 ± 141	+135	±32
45-54	83	633 ± 110	58	509 ± 140	+124	±22
55-64	123	574 ± 126	64	514 ± 88	+ 60	±16
65-74	116	550 ± 145	36	469 ± 107	+ 81	±22
75-84	30	483 ± 99	12	498 ± 164	— 15	±59
85-94	5	415	1	550	.....	....
Total.....	402	575 ± 141	218	499 ± 120		

Slight coronary arteriosclerosis = scattered intimal atheromatous plaques without stenosis of the lumen.
Moderate coronary arteriosclerosis = many atheromatous or calcified plaques with no significant stenosis of the lumen.
Marked coronary arteriosclerosis = atheromatous and calcified sclerotic plaques with stenosis of any degree or occlusion of the lumen of the vessel.

TABLE 2.—*Comparative Weights of Hearts of Hypertensive Females with Moderate and Marked Coronary Arteriosclerosis and of Those with Slight or No Coronary Arteriosclerosis*

Age Group	Moderate and Marked Coronary Arteriosclerosis: Cases	Moderate and Marked Coronary Arteriosclerosis: Mean Heart Weight with Standard Deviation	Slight or No Coronary Arteriosclerosis: Cases	Slight or No Coronary Arteriosclerosis: Mean Heart Weight with Standard Deviation	Difference Between Means	Standard Error of Difference Between Means
15-24	1	390	3	358	.....	....
25-34	5	528 ± 106	11	478 ± 110	+ 50	±59
35-44	24	571 ± 124	48	468 ± 67	+103	+27
45-54	50	527 ± 126	47	459 ± 141	+ 68	±27
55-64	60	487 ± 65	28	430 ± 119	+ 57	±24
65-74	37	460 ± 124	18	398 ± 136	+ 62	±38
75-84	24	423 ± 223	10	335 ± 69	+ 88	±50
85-94	7	357	1	300	.....	....
Total.....	214	491 ± 133	166	441 ± 119		

possible that coronary arteriosclerosis plays some part in the development of cardiac hypertrophy in prolonged hypertension. Since stretching of the muscle in diastole is the ultimate stimulus to hypertrophy, it may be that disease of the coronary arteries can reduce blood flow in that

system to a degree which favors diastolic stretching without at the same time interfering with sufficient nutrition to permit of hypertrophy.

There are, however, certain elements in the analysis which may interfere with the precision of the results. There is no sharp dividing

TABLE 3.—*Comparative Weights of Hearts of Hypertensive Males with Marked Coronary Arteriosclerosis and of Those with Slight or No Coronary Arteriosclerosis*

Age Group	Marked Coronary Arteriosclerosis: Cases	Marked Coronary Arteriosclerosis: Mean Heart Weight with Standard Deviation	Slight or No Coronary Arteriosclerosis: Cases	Slight or No Coronary Arteriosclerosis: Mean Heart Weight with Standard Deviation	Difference Between Means	Standard Error of Difference Between Means
15-24	..	.....	1	375	.....	....
25-34	4	592 ± 95	14	493 ± 94	+ 99	....
35-44	22	624 ± 109	32	491 ± 141	+133	±33
45-54	42	634 ± 172	58	509 ± 140	+125	±33
55-64	65	585 ± 125	64	514 ± 88	+ 71	±19
65-74	68	565 ± 131	36	469 ± 107	+ 96	±24
75-84	24	479 ± 117	12	498 ± 164	− 19	....
85-94	4	431 ± 37	1	550	.....	....
Total.....	229	578 ± 142	218	499 ± 120		

TABLE 4.—*Comparative Weights of Hearts of Hypertensive Females with Marked Coronary Arteriosclerosis and of Those with Slight or No Coronary Arteriosclerosis*

Age Group	Marked Coronary Arteriosclerosis: Cases	Marked Coronary Arteriosclerosis: Mean Heart Weight with Standard Deviation	Slight or No Coronary Arteriosclerosis: Cases	Slight or No Coronary Arteriosclerosis: Mean Heart Weight with Standard Deviation	Difference Between Means	Standard Error of Difference Between Means
15-24	..	.....	3	358	.....	....
25-34	1	600	11	478 ± 110	.....	....
35-44	10	636 ± 132	48	468 ± 67	+168	±43
45-54	23	544 ± 141	47	459 ± 141	+ 85	±36
55-64	28	510 ± 97	28	430 ± 119	+ 80	±29
65-74	21	473 ± 221	18	398 ± 136	+ 75	±58
75-84	18	423 ± 103	10	335 ± 69	+ 88	±33
85-94	5	387 ± 88	1	300	+ 87	....
Total.....	106	503 ± 154	166	441 ± 119		

line between marked, moderate and slight sclerosis either in the coronary arteries or elsewhere. A diagnosis of the presence or absence of sclerosis in the major divisions may not necessarily reflect the condition of the finer twigs of that arterial tree. As ordinarily practiced in the autopsy room, the determination of cardiac weight is not exact. The heart

weight: body weight ratio could not be established in this study, because the dead body was weighed in only one of the hospitals. Even had this been done in all, the lack of precision of the other factors would place some doubt on the validity of the ratio.

TABLE 5.—*Comparative Weights of Hearts of Hypertensive Males with Moderate Coronary Arteriosclerosis and of Those with Slight or No Coronary Arteriosclerosis*

Age Group	Moderate Coronary Arteriosclerosis: Cases	Moderate Coronary Arteriosclerosis: Mean Heart Weight with Standard Deviation	Slight or No Coronary Arteriosclerosis: Cases	Slight or No Coronary Arteriosclerosis: Mean Heart Weight with Standard Deviation	Difference Between Means	Standard Error of Difference Between Means
15-24	..	.....	..	.....	.....	....
25-34	3	565	14	493 ± 94	+ 72	....
35-44	16	628 ± 125	32	491 ± 141	+137	±40
45-54	41	615 ± 154	58	509 ± 140	+106	±30
55-64	58	554 ± 84	64	514 ± 88	+ 40	±15
65-74	48	527 ± 109	36	469 ± 107	+ 58	±24
75-84	6	496 ± 81	12	498 ± 164	— 2	....
85-94	1	350	1	550	.....	....
Total.....	173	565 ± 127	217	499 ± 120		

TABLE 6.—*Comparative Weights of Hearts of Hypertensive Females with Moderate Coronary Arteriosclerosis and of Those with Slight or No Coronary Arteriosclerosis*

Age Group	Moderate Coronary Arteriosclerosis: Cases	Moderate Coronary Arteriosclerosis: Mean Heart Weight with Standard Deviation	Slight or No Coronary Arteriosclerosis: Cases	Slight or No Coronary Arteriosclerosis: Mean Heart Weight with Standard Deviation	Difference Between Means	Standard Error of Difference Between Means
15-24	1	390	3	358	+32	....
25-34	4	442 ± 127	11	478 ± 110	—36	....
35-44	14	524 ± 131	48	468 ± 67	+56	±37
45-54	33	522 ± 124	47	459 ± 141	+63	±30
55-64	32	451 ± 84	28	430 ± 119	+21	±27
65-74	16	419 ± 117	18	398 ± 136	+21	±43
75-84	6	414 ± 175	10	335 ± 69	+79	....
85-94	2	282 ± 17	1	300	....	....
Total.....	108	448 ± 193	166	441 ± 119		

SUMMARY

Within the limitations of this sort of statistical study, the results suggest that arteriosclerosis of the coronary arteries may play a part in the genesis of cardiac hypertrophy in patients with prolonged hypertension.

Case Reports

LIPOID STORAGE CELLS IN LYMPHOSARCOMA

SIEGFRIED TANNHAUSER, M.D., TUCSON, ARIZ.

The occurrence of large pale "storage cells" in inflammatory or hyperplastic conditions of the reticuloendothelium has been reported even outside the typical storage diseases, such as Gaucher's splenomegaly, Niemann-Pick disease and Hand-Schüller-Christian disease. Such cells have been observed in rickets [1] and tuberculosis; [2] they occur occasionally in chronically inflamed lymph nodes.[3] Herzog and associates [4] reported a case of reticuloendotheliosis with lipoid storage (cholesterol), which was interpreted as showing a possible transition between Hand-Schüller-Christian disease and malignant lymphoblastoma.

The occurrence of these "storage cells" in true neoplastic diseases is rare. I report briefly a case of lymphosarcoma of the large intestine with "storage cells" present in abundance.

REPORT OF A CASE

A white man 42 years of age complained for some time of weakness, constipation and loss of weight. From time to time he felt a dull aching in the left upper abdominal quadrant. In April 1940 there occurred a sudden hemorrhagic diarrhea, which recurred the following day. Roentgen examination after a barium sulfate enema showed a circular filling defect approximately 10 cm. distal to the splenic flexure, occupying nearly the whole lumen of the colon. At operation there was found in the descending colon, in the area suggested by the roentgenogram, a pedunculated tumor protruding into the intestinal lumen. The surrounding intestinal tissue showed no infiltrations; no other growth or metastasis could be detected. The tumor was removed, and the patient made an uneventful recovery.

The gross specimen was a firm tumor of the shape of a small tomato, measuring about 4 by 6 cm. It was well encapsulated except for a slightly ulcerated area in the surface at the top. The encapsulation was particularly marked in the region of the broad peduncle. Cross section showed a whitish firm uniform tissue; however, in the vicinity of the ulceration there were reddish streaks and yellowish zones, apparently representing hemorrhages and necrotic areas.

Microscopically, the major part of the tumor consisted of a cellular tissue with scanty stroma. The cells were for the most part lymphocytes with narrow cytoplasm and of circular or oval shape. The nuclei of the lymphocytes were moderately deeply stained and occasionally showed a radiating arrangement of the chromatin. In other areas the nuclei as well as the cell bodies were markedly

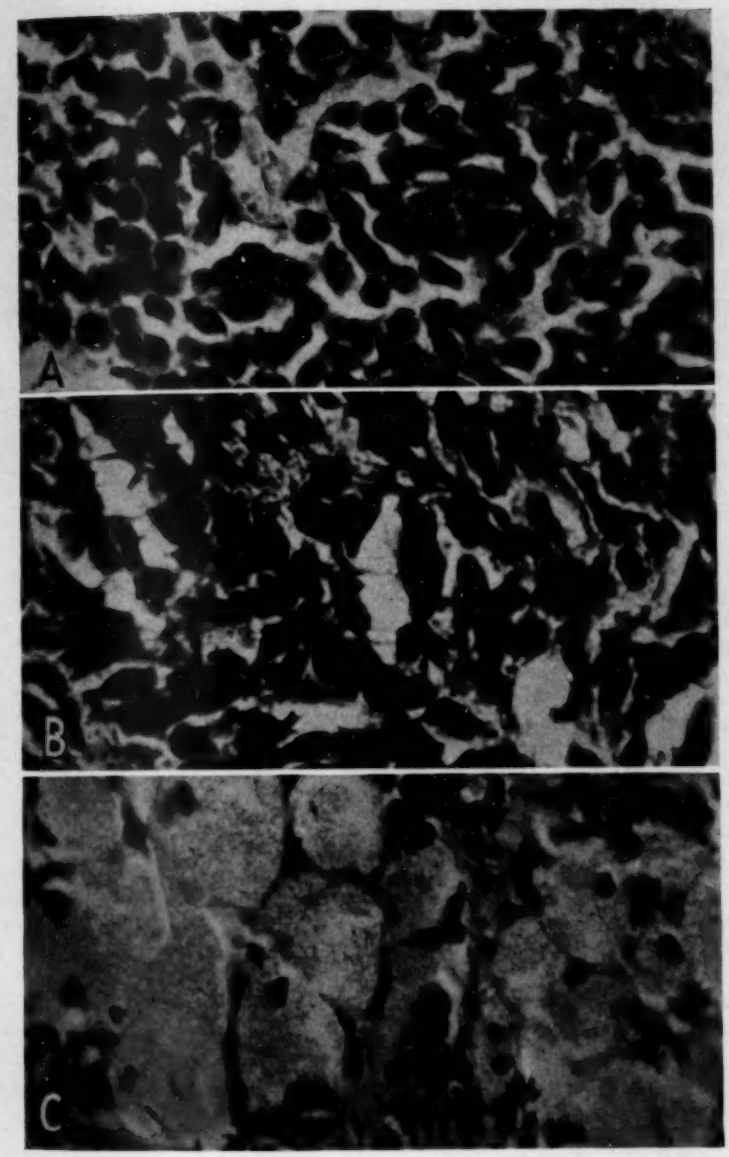
From the Department of Pathology, Desert Sanatorium.

1. Stilling, cited by Nägeli, O.: Blutkrankheiten und Blutdiagnostik, Berlin, Julius Springer, 1931, p. 556.

2. Bovaird, W. J.: Am. J. M. Sc. **120**:377, 1900.

3. Ribbert, cited by Nägeli, O.: Blutkrankheiten und Blutdiagnostik, Berlin, Julius Springer, 1931, p. 557.

4. Hertzog, A. J.; Anderson, F. G., and Beebe, G. W.: Arch. Path. **29**:120, 1940.



Lymphosarcoma of the colon. *A*, area giving the appearance of lymphocytoma. *B*, area giving the appearance of reticulum cell sarcoma. *C*, storage cells in the tumor.

unequal in size; there were giant nuclei and cells showing several nuclei, also alternation of hyperchromatic and hypochromatic nuclei. Mitoses were not abundant. Occasionally cells of the plasma cell type were seen, but they were not predominant anywhere. Still other parts of the tumor showed larger cells, each with polyhedral cytoplasm and a pale vesicular nucleus. These cells were interconnected by fine fibrils which extended from their corners. In some areas there was a distinctly reticular structure with formation of sinusoids. There was abundant vascularization, particularly of the hemorrhagic regions, which showed ectatic blood vessels. One area showed a plug of tumor tissue which had grown into a small dilated blood vessel. A fibrous capsule composed of several layers of connective tissue surrounded the tumor. In a section of the ulcerated area, the tumor tissue perforated this fibrous capsule from below and infiltrated the mucosa for a considerable distance. The tumor cells compressed the intestinal glands and in some places formed the lining of the intestine. In this section extensive hemorrhages involved the mucosa, the fibrous capsule and the underlying tumor. It should be noted that the glands of the mucosa were generally well preserved except in the hemorrhagic or infiltrative areas, where they were scarce and shorter than normal. The glandular elements disclosed no evidence of carcinoma.

Some areas of the tumor showed increased development of fibrous tissue with a relatively scarcity of the cells between; still other areas stained poorly or were even frankly necrotic.

The most conspicuous feature in this tumor were large pale cells, between 20 and 40 microns in diameter, occurring either single or in large accumulations. Their cytoplasm showed a finely reticulated, foamy structure, their nuclei were generally small and rather pyknotic; some of the cells had larger, vesicular nuclei. These cells resembled closely the "storage cells" in Niemann-Pick disease and related conditions. In places these cells were abundant and constituted the main bulk of the tumor; this was particularly true of the vicinity of the hemorrhagic and necrotic areas. Chemical examination of the nature of the lipoid substance in these cells was not possible due to the scarcity of material and the regional occurrence of these cells within the tumor. The sections were embedded in paraffin and stained with hematoxylin–eosin.

The diagnosis was lymphosarcoma of the large intestine with features of reticulosarcoma.

COMMENT.

The case reported presents as an outstanding feature the abundant occurrence of large lipoid "storage cells" in lymphosarcoma. Besides, it emphasizes the variation of cell and structural types which may compose this kind of tumor. Epstein [5] shows how the morphologic appearance of such cells with ordinary staining technics may be of help to differentiate between the phosphatide-storing cells of Niemann-Pick disease and the kerasin-storing cells of Gaucher. The cells in the present case appear similar to those in Niemann-Pick disease on account of their finely reticulated structure.

Lymphosarcoma has always had a doubtful place in the sarcoma group. Its possible relationship to the inflammatory conditions of the lymph nodes has often been shown (Ewing [6]), and the transition from these conditions to the picture seen in truly malignant new growths may be very gradual.

5. Epstein, E.: Virchows Arch. f. path. Anat. **281**:152, 1931.

6. Ewing, J.: Neoplastic Diseases, ed. 3, Philadelphia, W. B. Saunders Company, 1928, pp. 421-423.

The reticuloendotheliosis reported by Hertzog and associates,[4] in which "storage cells" were equally well shown, impresses one as being predominantly hyperplastic with tumor-like qualities. In contradistinction, the condition which I have reported is truly a malignant one with some properties of hyperplasia of the reticuloendothelium, as evidenced in the presence of "storage cells."

The occurrence of areas which show lymphocytes of variegated types, and reticulum cells, with formation of sinusoids, within the same tumor deserves a short comment. There has been of late a tendency to set up the reticulum cell tumors as a separate group within the classification of tumors. This distinction has been of definite value for the classification of hyperplastic and tumor-like conditions, such as mycosis fungoides and reticulosis of the skin, which so far have been difficult to classify. Attention was called to the tumor-forming potency of the reticulum cells and their widespread distribution throughout the body.

In lymphosarcoma, however, and in some related tumors a sharp distinction is not always feasible and from a theoretic standpoint not justifiable. Ewing [6] discussed extensively the complicated conditions in the hyperplastic and tumor-like diseases of the lymphoid system and spoke of "reticulum cell type lymphosarcoma," which he distinguished from malignant lymphocytoma. He stated that "it would greatly simplify the questions of histogenesis if separation of the two types of cells were established. Most pathologists, however, the writer among them, are unable to satisfy themselves that the reticulum cells during the course of inflammatory and neoplastic changes may not assume the form of lymphocytes."

SUMMARY

A case of lymphosarcoma of the colon is reported which was remarkable from two standpoints: First, the tumor showed the occurrence of great numbers of lipoid storage cells; second, the histologic picture presented features of malignant lymphocytoma and of reticulum cell sarcoma within the same tumor.

SPONTANEOUS RUPTURE OF THE POSTERIOR PAPILLARY MUSCLE OF THE HEART

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Spontaneous rupture of a papillary muscle of the heart has long been recognized as a definite pathologic entity. However, the condition is so rare and so lacking in distinctive clinical features that it defies diagnosis except at the autopsy table.

The first report of a case to appear in the literature was that by Merat,[1] in 1803. In 1935 Stevenson and Turner [2] reviewed the literature and summarized the data on each of the 19 cases which had been recorded up to that time and reported a case of their own. Since then Hausen-Faure and Hasenjäger [3] have reported 2 cases and Moragues [4] 1. Therefore, to date 23 cases have been described in the literature. In view of the infrequency of the lesion it seems worth while to report another case.

REPORT OF CASE

A 68 year old, unemployed white man entered the Kings County Hospital on Jan. 13, 1939 at 2:30 p. m. He was acutely ill and complained of pressure and tightness in the chest. Because of his extreme condition, only a brief history was obtainable. On the morning of admission, following an argument with his landlord, he collapsed and began to have a sense of tightness in the chest. This persisted without remission, and at the time of admission the pain was localized over the precordium. He also complained of shortness of breath. His daughter stated that he had suffered from a "heart attack" one week before, at which time he "almost collapsed." For the past week he had been expectorating bloody sputum. He had had a cough for one month.

Examination on admission showed an elderly white man who was acutely ill, cyanotic and dyspneic and whose skin was cold and clammy. The pulse rate was 120, the respiration rate 34, the temperature 95 F. and the blood pressure 70 systolic and 60 diastolic. The pupils were round and equal and reacted to light and in accommodation. The chest was emphysematous, with an increased anterior-posterior diameter. Percussion elicited hyperresonance throughout, and the breath sounds were of an exaggerated vesicular type. There were moist, crepitant rales at both bases. The left border of the heart was in the anterior axillary line, with the apex beat in the fifth interspace. There was tachycardia with a regular rhythm. The heart sounds were of good quality, and no murmurs were heard. The abdomen

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From the Department of Pathology, Kings County Division, Long Island College of Medicine.

1. Merat: J. de méd., chir., pharm. **6**:587, 1803; cited by Stevenson and Turner.[2]

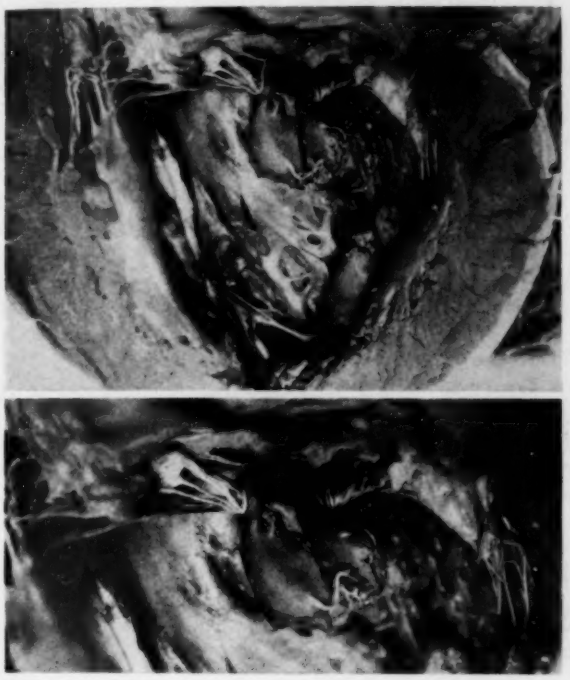
2. Stevenson, R. R., and Turner, W. J.: Bull. Johns Hopkins Hosp. **57**: 235, 1935.

3. Hausen-Faure, G., and Hasenjäger, E.: Klin. Wchnschr. **17**:540, 1938.

4. Moragues, V.: Am. Heart J. **17**:106, 1939.

presented some spasticity and tenderness in the right upper quadrant, but no masses or organs were palpable. There was no edema of the genitalia or of the extremities. A diagnosis of coronary occlusion was made.

His course was rapidly downhill. Soon the heart sounds became weaker, and rales were heard in the axillary regions. The abdomen remained spastic and tender in the right upper quadrant, and it was believed that the resistance was probably due to enlargement of the liver. At 5 p. m. the blood pressure was still 70 systolic and 60 diastolic and the pulse rate 118. For the first time there was heard what several observers thought to be a friction rub over the fourth interspace, just to the left of the sternum. The rhythm was suggestive of a gallop. At



Upper: Rupture of posterior papillary muscle. Lower: Twisted chordae tendineae of mitral valve.

7 p. m. the pulse was slower but irregular. The friction rub over the precordium was louder than previously. At 9 p. m. the pulse was thready, with a rate of 100, and the blood pressure was unobtainable. The patient was sleeping, and the respirations were deep and labored. He died at 1:25 a. m., respirations and heart sounds ceasing simultaneously.

An electrocardiogram taken at 4:45 p. m. was reported as showing right axis deviation, first degree heart block, sinus tachycardia and myocardial infarction (T_3 type).

Autopsy.—External examination revealed nothing unusual. In each pleural cavity there was approximately 500 cc. of clear straw-colored fluid, and one noted a few

old, fibrous adhesions. The pericardial sac was covered with a thick layer of adipose tissue and contained about 50 cc. of clear yellow fluid. There were no pericardial adhesions or exudate.

The heart weighed 580 Gm. It was covered by a smooth and glistening epicardial surface. Subepicardial adipose tissue followed the course of the coronary vessels. On external palpation, there was an area over the anterior and posterior aspects of the left ventricle that was softer in consistency and had a more homogeneous, deeper red coloration than the surrounding myocardium. Only postmortem blood clots filled the chambers of the ventricles and atriums. Cardiac hypertrophy and dilatation were manifested by thickening and flattening of the trabeculae carneae and papillary muscles. Some increase of focal myocardial fibrosis was demonstrated by a tangential section of the myocardium. The leaflets of the tricuspid and mitral valves were somewhat thickened by yellow plaques near the bases of the valves and by fibrosis of the edges, with a moderate folding of the free edges. The chordae tendineae were thickened and attached at the free edge of the thickened valve leaflets. The cusps of the pulmonic and aortic valves were thin and velamentous. The posterior papillary muscle to the mitral valve was ruptured and completely separated (see upper part of figure). The torn ends of the muscle were rounded, roughened, gray and translucent. The stump measured 1.5 cm. in length. The portion of the severed muscle attached to the chordae tendineae had become entangled between the valve leaflets and the attached chordae tendineae (lower part of figure). In the wall of the ventricle, in the area of this papillary muscle, there were hemorrhage, necrosis and softening typical of an infarct. The right and left coronary arteries were tortuous and had a pipestem appearance. The left coronary artery showed organized and recent thrombi in all of its branches. The right coronary artery was patent, but its lumen was narrowed by numerous yellow atheromatous plaques.

Microscopic examination of the heart showed the muscle nuclei to be bizarre in size and shape, characteristic for hypertrophied muscle fibers. Scattered throughout the myocardium were diffuse areas of fibrosis. Some sections also showed regions of recent infarction. Evidence of early organization was seen in the presence of fibroblasts and young, newly formed capillaries. This was particularly noticeable, especially at the tip of the ruptured papillary muscle. The coronary arteries showed atheromatous thickenings which greatly decreased the caliber of the vessels' lumens. Fresh thrombi and organized and canalized thrombi occluded the lumen of the left coronary artery.

The gross and histologic changes in the remaining organs were not remarkable except that there was excessive pulmonary edema.

COMMENT

Stevenson and Turner [2] and Moragues [4] have brought out that in the majority of the cases reported the rupture of a papillary muscle was secondary to arteriosclerosis of the coronary arteries and myocardial infarction. The muscle most commonly involved, as in our own case, was the posterior papillary muscle to the mitral valve. In only 2 cases was a muscle to the tricuspid valve affected. Of the ruptures which occurred on the left side of the heart, 1 involved both the anterior and the posterior muscles, 4 occurred in anterior muscles, and 12, including our own, were located in posterior muscles. In 4 cases in which the rupture occurred in the left ventricle the exact muscle was not indicated.

Only a few of the authors who have recorded cases in the literature have made any positive statement as to the presence of cardiac hypertrophy; none has mentioned the condition of the valve leaflets and the chordae tendineae. It is believed that in our case the greatly thickened mitral valve and shortened chordae tendineae together with the hypertrophied myocardium were factors that brought about excessive strain on the necrotic papillary muscle so as to cause its rupture.

Moragues [4] stated that no one has ever correctly diagnosed rupture of a papillary muscle clinically. This is probably because of its comparative rarity and the similarity of the clinical picture to that of an ordinary coronary occlusion. The history, in the large majority of cases, has been one of sudden onset of severe precordial pain, sometimes radiating to the left shoulder and arm and frequently associated with dyspnea and cyanosis. Usually the rupture appears in the course of moderate cardiac disease in an elderly person. The suddenness of onset and the severity of the pain are so uniformly present in the reported cases as to be considered typical. In the face of the acute mitral insufficiency resulting from this accident, pulmonary edema is immediate and progressive, dyspnea becomes increasingly marked, and cyanosis appears. Ours is the only report of a case presenting an electrocardiogram. As we should expect, the findings were typical of coronary occlusion, myocardial infarction and heart block. Sudden death, or death within a few hours, is the rule, although one patient is said to have lived for twenty months and another for ten months.

One clinical finding which seems to be significant is reported in association with several cases in the literature. This consists of the appearance of a murmur following the onset of the acute pain. In some cases the murmur is systolic; in some, diastolic, and in some it is of the to and fro type. The important thing is the appearance of a new and definite murmur immediately after the onset of symptoms. In our case several competent observers heard, some time after admission, a loud sound which had not been present previously and which they interpreted as a friction rub. In view of the patient's history and the fact that the ends of the torn muscle were partly organized, we set the time of the rupture as several hours before admission to the hospital. Consequently there was some discussion as to why this "friction rub" should appear suddenly following a delay from the apparent time of rupture. We have tried to explain this on the hypothesis that the ruptured muscle at first caused no unusual sounds, but as it became twisted and tangled in the chordae tendineae it set up the extraneous sound which was later heard. The other possibility is that the patient entered the hospital with coronary occlusion and myocardial infarction and that the actual rupture of the muscle occurred after admission to the hospital. In that case the organization of the torn ends must have developed in the course of a few hours.

SUMMARY

This is a report of another case of rupture of the papillary muscle following infarction of the posterior myocardial wall. It is believed that the rupture of the necrotic papillary muscle was the result of excessive strain caused by the hypertrophied myocardium and shortened chordae tendineae of the thickened mitral valve.

MALIGNANT MESONEPHROMA OF THE OVARY

J. A. Tuta, Ph.D., M.D., and J. E. Siebel, M.D., Chicago

Recently attention has been called to a group of ovarian tumors having certain distinctive histologic features, which have been classified by Schiller [1] as being of mesonephric origin. He collected 10 such tumors; 1 was of intraligamentous origin; 1 was associated with a dermoid, and 1, with an embryoma. Jones and Seegar [2] reported 6 more tumors of this group. Of the 16 tumors reported, 9 have been malignant.

The characteristic histologic features of the group consist of cystic spaces lined by flattened endothelial-like cells with projecting nuclei and papillary intracystic formations.

Popoff [3] discussed certain growths which have been called wolffian epithelioma, reports of which have appeared chiefly in the French literature. He reviewed the histologic characteristics and mentioned papillary formations but did not describe the characteristic cystic spaces with flattened endothelial-like cells.

REPORT OF CASE

A 49 year old housewife was admitted to the Grant Hospital of Chicago in December 1933, complaining of backache, constipation and leukorrhea. Menstruation had ceased one year before. She had never been pregnant. She was operated on, and a left ovarian cyst about the size of a grapefruit was removed. The cyst ruptured during the operative procedure. The specimen consisted of a piece of edematous ovarian tissue with deposits of soft white homogeneous tissue, up to 15 mm. in diameter. Attached to the ovary was an opened cyst, 9 cm. in diameter, with a fibrotic wall and a smooth outer surface. It was thought at the time that the solid white tissue might be classified as a fibroepithelial tumor of the Brenner type and that it was suggestive of malignancy.

In December 1936 the patient began having pelvic pain and was given a series of roentgen ray treatments, which relieved the pain for a few weeks. In February 1937 she was operated on a second time. A lobulated tumor mass, 10 by 5 by 4 cm., was removed from the left broad ligament. It was adherent to the descending colon. The surfaces made by sectioning the tumor mass were purple gray mottled with irregular yellow-gray areas. Reexamination in 1939 suggested a further pelvic recurrence.

Microscopic Description of Tumor.—The tumor tissue removed in 1933 shows groups of polyhedral cells with vesicular cytoplasm, separated by a few connective tissue fibrils. Irregular cyst formation is found in some of the cellular areas. In other areas there is more abundant stroma, in which are cysts of varying sizes lined by a single layer of cuboid cells or flattened endothelial-like cells with pro-

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From the Grant Hospital of Chicago and the Department of Pathology of the University of Illinois College of Medicine.

1. Schiller, W.: Am. J. Cancer **35**:1, 1939.

2. Jones, H. W., and Seegar, G. E.: Am. J. Obst. & Gynec. **39**:322, 1940.

3. Popoff, N. W.: Arch. Path. **9**:31, 1930.

jecting nuclei (fig. 1*A*). Other areas have a loose, edematous structure. There is an occasional cell with an enlarged and hyperchromatic nucleus. In small areas the nuclei appear pyknotic. In some of the cystic spaces there are occasional

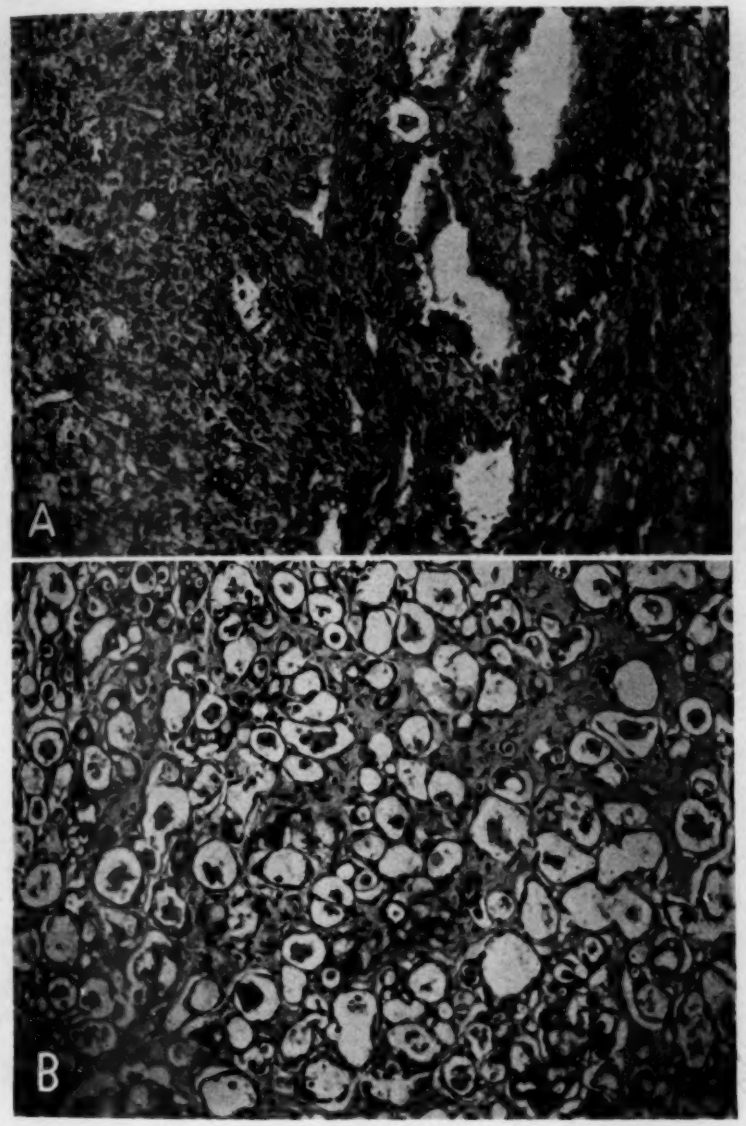

Fig. 1.—*A*, tumor removed in 1933, showing irregular cystic spaces with projecting nuclei and also groups of cells with occasional small cystic spaces; × 120. *B*, tumor removed in 1937, showing cystic spaces lined by endothelial-like flattened cells; × 120.

small papillary formations. Some of the granular material within the cystic spaces and occasionally some of the cells take the mucicarmine stain.

The tumor tissue which was removed in 1937 contains numerous irregular spaces of varying sizes. Many of the spaces are lined with cells which have

Fig. 2.—Tumor removed in 1937, showing glomerular-like structures; × 187.5.

projecting nuclei and small amounts of cytoplasm. Some of the spaces are lined by markedly flattened cells, which resemble endothelium of the vascular or lymphatic system (fig. 1*B*). There are irregular papillary formations, some of which almost fill the cystic spaces. They are composed of groups of medium-

sized polyhedral cells, some of which are hyperchromatic. Figure 2 shows structures which resemble glomeruli. The papillary processes which project into the smaller cystic cavities show only very occasional delicate connective tissue or capillary formation with the Mallory phosphotungstic acid–hematoxylin and Van Gieson stains. Some of the larger cystic cavities contain complex papillary processes, showing a connective tissue core and a few capillaries. There are also small cystic spaces in some of these papillary formations. The cystic cavities containing the papillary processes are lined for the most part by flattened cells with projecting nuclei. The material in some of the cystic spaces takes the muci-carmine stain. Large areas of necrosis are present.

In a separate nodule, taken from the pelvis adjacent to the large tumor mass, there are complex papillary intracystic formations, containing connective tissue

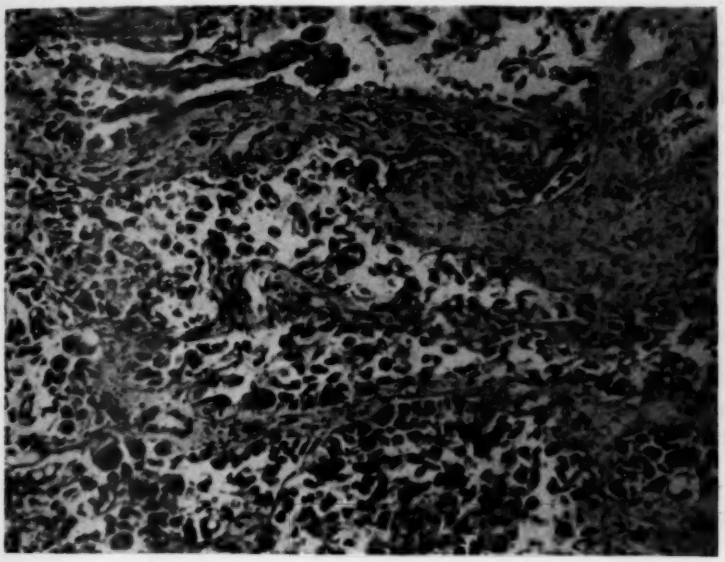

Fig. 3.—Separate tumor nodule removed in 1937, showing marked nuclear anaplasia; × 120.

and capillaries. The nuclei show greater variation in size and hyperchromatism (fig. 3). Many of the cells have several prominent nucleoli. At the periphery of the nodule there is compressed connective tissue, which is infiltrated by very anaplastic tumor cells. There are only a few rounded cystic spaces, and these are lined by cuboidal epithelium. The intracystic material does not take the muci-carmine stain.

COMMENT

A comparison of the tissue removed in 1933 with that of 1937 shows in both areas having the same characteristic histologic pattern, i. e., cystic spaces lined by flattened endothelial-like cells with projecting nuclei and scant cytoplasm. Slight papillary processes are only occasionally seen in the tissue removed at the first operation. The slight nuclear irregularities are suggestive of early malignant changes.

In contrast, the tissue removed at the recurrence is definitely malignant. Papillary formations are a prominent feature, occasionally resembling glomeruli. Whether these are true attempts at the formation of glomeruli is a point of conjecture. The most malignant-appearing area has the most complex papillary formations and an absence of the small cystic spaces with the characteristic flattened cells.

Jones and Seegar [2] found similar characteristic histologic features in their group of cases. They discussed the resemblance of certain microscopic areas to mesonephric glomerular structures, cautioning their readers to bear in mind, however, that the mesonephric origin was not definitely proved.

If the papillary formations represent an attempt to differentiate and form mesonephric glomerular structures, one might expect them to be present in the more benign tissue from the first operation. However, in that tissue the papillary processes are rare, while in the recurrence they are much more prominent.

The evidence which has been offered for the mesonephric origin of the ovarian mesonephroma was found in a comparison of the structure of the ovarian tumor with that of tumors originating in the broad ligaments where embryonic wolffian remnants are found. The first tumor of the latter type which Schiller [1] described was extraovarian and intraligamentous in origin and had a structure similar to that described for those of intraovarian origin. Hansmann and Budd [4] made an extensive study of the retroperitoneal tumors of mesonephric origin and compared them with tumors found in adult urogenital organs.

In a cervical adenoma of Gartner's duct reported by Wolfe,[5] the type of epithelium covering the papillary processes was flattened with a scant amount of cytoplasm.

Schiller [1] and Jones and Seegar [2] commented on the preponderance of tumors involving the right ovary. Only 2 of 16 reported have been found definitely on the left side. The present tumor was confined to the left side. Popoff [3] cited several observations recorded in the literature in which embryologic defects in animals and the human species were found more often on the right side than on the left. Seegar [6] stated that the dysgerminoma is more commonly found involving the right ovary. The number of cases so far reported is not sufficient to enable one to attach much significance to the predominance of tumors on the right side.

SUMMARY

A malignant cystic ovarian tumor has been classified, because of its characteristic histologic structure, as belonging in a group probably of mesonephric origin. A comparison of the relatively benign primary tumor and the more malignant recurrence showed that both had a common characteristic histologic structure.

4. Hansmann, G. H., and Budd, J. W.: J. A. M. A. **98**:6, 1932.

5. Wolfe, S. A.: Am. J. Obst. & Gynec. **39**:312, 1940.

6. Seegar, G. E.: Arch. Surg. **37**:697, 1938.

Laboratory Methods and Technical Notes

NOTE ON A SIMPLE METHOD FOR ZENKERIZING PARAFFIN SECTIONS OF FORMALDEHYDE-FIXED TISSUES

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With only formaldehyde-fixed tissues at hand, it is often desirable to obtain certain stains which ordinarily require fixation in Zenker's solution. Several methods of mordanting or zenkerizing tissues fixed in solution of formaldehyde U. S. P. are offered in the literature and in certain texts on histologic technic. In general, these are too involved and take too long for common use. The method to be described here has been found simple, rapid and reliable. Mallory's aniline blue connective tissue stain, Mallory's phosphotungstic acid–hematoxylin stain and Giemsa's stain have been employed. Sections do not come off the slides even when exposed to the mordant for periods longer than the suggested time.

METHOD

Mounted paraffin sections of tissue fixed in solution of formaldehyde U. S. P. are run through xylene and successive dilutions of alcohol down to water.

They are then mordanted at room temperature for four hours in a solution composed of Zenker's fluid and concentrated nitric acid in a concentration of 5 per cent (Zenker's fluid, 95 parts; concentrated nitric acid, chemically pure, 5 parts). This solution is made fresh as needed. Heat is generated on preparing this solution, and it should then be cooled to room temperature before being used.

They are washed in running tap water to remove all traces of the mordant. Usually fifteen minutes' washing will suffice.

Although no precipitate is formed in the tissues, the use of an iodine solution and thiosulfate results in better differentiation and finer detail in staining.

The sections are now in a condition similar to that of Zenkerized sections with the precipitate removed. From this point on, the usual staining technics are followed.

The results in the great majority of sections are superior to those obtained with other methods of mordanting and are sufficiently close to those obtained with Zenker fixation to warrant the use of this method in the pathology laboratory of the University of California Hospital and Medical School.

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General Reviews

MALFORMATIONS OF THE LUNG

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AND
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ST. LOUIS

The general use of roentgenography in the diagnosis of conditions of the chest and the increased scope of thoracic surgery necessitate a certain familiarity with the more unusual forms of pulmonary disease. Among the rarer findings we may list a variety of malformations which clinically or on the roentgen plate may be easily mistaken for the common types of pulmonary disease or may, if not thought of at all, baffle the diagnostician completely.

Notwithstanding the rarity of these abnormalities, a sufficient number of specimens have been described to warrant a review and a tentative classification based on recognition of the underlying developmental disturbance. Some of the most informative descriptions date back to the preroentgen era, when these finding were encountered mainly at autopsies. At the turn of the century, when embryology still occupied a most prominent place in medical science, teratologic specimens were usually submitted to a painstaking analysis, and therefore one finds in these earlier publications many valuable references to the normal and to the faulty development of the respiratory organs. The summaries given by Eppinger,[1] Schneider,[2] Bert and Fischer,[3] and Koontz,[4] are interesting not only because they refer to rare and bizarre cases but also because they group the material according to the prevailing teratologic knowledge, which basically has remained unchanged.

GENERAL COMMENTS ON THE GENESIS OF THE LUNGS

We may speculate that the occurrence of developmental anomalies in the lung is favored by the phylogenetic newness of this organ and the enormous growth it must undergo in the embryo before its func-

From the Laboratory of the Jewish Hospital.
This study was aided by the David May–Florence May Fund.

1. Eppinger, H.: Ergebn. d. allg. Path. u. path. Anat. **8**:267, 1902.

2. Schneider, P., in Schwalbe, E.: Die Morphologie der Missbildungen des Menschen und der Tiere, Jena, Gustav Fischer, 1912, vol. 3, pt. 2, chap. 8.

(Footnotes continued on next page)

tional form is achieved. Beginning with its embryonic vestiges, two different pulmonary anlagen can be recognized: an epithelial one, which plays the main role during the formative period of the organ, and a mesenchymal one, the importance of which increases when the functional stage is approaching. The epithelial anlage appears as a longitudinal groovelike evagination of the anterior wall of the foregut. Separation from the esophagus is accomplished by fusion of the two opposite edges of the evagination, proceeding in a caudal-cranial direction (formation of the tracheoesophageal septum). At the cranial end, the septum stops short of accomplishing a complete separation, leaving a communication with the esophagus, the aditus laryngis. In this stage the epithelial lung anlage forms a tubular sac descending in front of the foregut and communicating with it. This primitive structure represents the beginnings of three organs of the respiratory tract: the larynx at the upper, communicating end; the trachea in the intermediate section, and the lung at the lower, blind pole. The latter soon becomes bifid, with the projections reaching into the right and left cavities of the chest. These two branches, called stem bronchi, develop, according to prevailing opinion, into the bronchial tree by arborization and downgrowth. Heiss,[5] however, expressed the belief that not arborization but infolding and septum formation are the developmental mechanics responsible for the progressive differentiation of the organ. He therefore rejects the term and the conception of stem bronchi. For him the large bronchi do not represent primordial structures, preceding all other parts of the lung; the embryonic "stem bronchus" is the periphery of the lung, from which the connecting and the terminal structures of the lung parenchyma are formed by a continuous folding in. However, the generally accepted explanation for the development of the lungs is that a progressive monopodial branching takes place from the right and left stem bronchi and that the new parts of the organs are formed by everting sprouts (Flint;[6] Ekehorn[7]). The bronchi grow into the right and left thoracic cavities, where the bilateral mesenchymal anlagen furnish the material for a very vascular stroma.

No unanimity exists about the microscopic anatomy of the end points of the tracheobronchial duct system, the alveoli. At first it seemed quite natural to assume that the alveoli should be lined by a continuation of the entodermal epithelium of the bronchial mucosa (Kölliker[8]). However, up to the present time, anatomists have disagreed as to whether

3. Bert, P., and Fischer, B.: Frankfurt. Ztschr. f. Path. **6**:27, 1911.
4. Koontz, A. R.: Bull. Johns Hopkins Hosp. **37**:340, 1925.
5. Heiss, R.: Ergebn. d. Anat. u. Entwcklngsgesch. **24**:244, 1924.
6. Flint, J. M.: Am. J. Anat. **6**:1, 1906.
7. Ekehorn, G.: Ztschr. f. Anat. **62**:271, 1921.
8. von Kölliker, A.: Entwicklungsgeschichte des Menschen, ed. 2, Leipzig, Wilhelm Engelmann, 1879, p. 867.

epithelial cells, or at least their derivatives, can be distinguished in the alveolar walls.[9] The epithelial cells are easily recognized in the fetus, and in the case of adult specimens of cold-blooded animals sections stained by silver methods are quite suggestive of them. In the higher animals, it is true, the conversion of the lungs into a functioning organ coincides with the effacement of the epithelial elements in the terminal extensions of the parenchyma. A majority of anatomists are at present of the opinion that respiration in the alveoli occurs without the participation of epithelial cells, which at best may persist as flattened out, not definitely recognizable disks. Some investigators believe that even these vestiges of the entodermal cells disappear from the alveoli.

DEFICIENT DEVELOPMENT OF THE LUNGS

The realization that the genesis of the lungs follows the pattern of a tubular-acinous duct system is essential for the understanding of most instances of congenital abnormality.

Teratogenesis may happen at any stage of the branching out of the primitive respiratory tract and its successor, the bronchial tree. An approximate determination of the time at which the growth process suffered interference or aberrance (*teratogenetischer Terminationspunkt*) should indeed form the basis for a scientifically significant classification of malformations. Such diagnosis, however, is only occasionally possible and is restricted to cases in which the largest units of the bronchial tree have been involved.

Since the investigations of Aeby, Narath and Flint,[10] it is known that the appearance of the two stem bronchi and the budding of side branches from them follow a rigid schedule. If one considers, furthermore, that, according to the prevailing opinion, the division of the lungs into lobes and the location of drainage areas within the lobes are determined by the topography of the bronchial branches, it becomes evident that inhibition or aberration in the sprouting of the bronchial tree may be reflected in severe defects or excesses of the tributary lung tissue.

Absence of Both Lungs.—The time when the primitive respiratory tube divides into the two stem bronchi is taken to be the fourth week, or when the fetus is about 5 mm. long. Therefore, any congenital defect which shows the bifurcation missing must have occurred before the time of appearance of the two stem bronchi. A case of this kind was

9. de la Croix, N. J.: Arch. f. micr. Anat. **22**:93, 1883. Fried, M. B.: Arch. Path. **3**:751, 1927. Rose, S. B.: ibid. **6**:38, 1928. Macklin, C. C.: J. Thoracic Surg. **6**:82, 1936.

10. (*a*) Aeby, C.: Der Bronchialbaum der Säugethiere und des Menschen, Leipzig, Wilhelm Engelmann, 1880. (*b*) Narath, A.: Der Bronchialbaum der Säugethiere und des Menschen, in Bibliotheca medica, Stuttgart, E. Nägele, 1901, no. 3, pt. A; cited by Flint [6] and by Kollmann.[14]

reported by Schmidt.[11] It concerned an 8 month premature baby, with larynx well developed and trachea not yet separated from the esophageal wall but possessing several islands of cartilage. The normally proportioned thoracic cavity contained only the heart, with no vestiges of the pulmonary veins. The pulmonary artery connected, as a large ductus Bottali, with the descending aorta. Pleural membranes were not found; the diaphragm was high. The arrest of bronchial development evidently took place before the primitive respiratory tube separated from the esophagus. A similar case was observed by Saito,[11] but in that instance separation of the trachea from the esophagus had taken place.

Agenesis of One Lung.—This condition has been reported in 36 instances.[12] A recent comprehensive survey was made by Hurwitz and Stephens;[13] an older one is contained in Schneider's[2] monograph. It is remarkable that in the majority of cases this malformation has been found compatible with life. Of the 36 subjects, 3 were stillborn, 5 lived less than one week, 12 lived less than six months, 1 lived two years and 1 five years, while 14 were between the fifth and the seventy-second year of age. The sexes were equally involved, and no predilection for one or the other side could be recognized. The stem bronchus of the aplastic lung varied in length. In some instances it was rudimentary; in others it had attained full length, and occasionally a nub of tissue at its end represented the agenetic lung. The majority of authors assumed an inherent deficiency of the lung anlage as the cause of the malformation. The fact that in several cases no interlobar fissure had formed in the opposite lung seems to support this view. Various attempts to explain the anomaly as due to mechanical interference with the growth process of the bronchial tree (persisting embryonic blood vessels, for example) have not been credited by embryologists.

Temporally, the developmental disturbance must have manifested itself after the division of the single primitive pulmonary organ into the two stem bronchi but before the stem bronchus developed its first side arms. According to Kollmann,[14] this would be in an embryo between 4.0 and 4.3 mm. in length; according to Grosser,[15] the respective lengths are 5.0 and 7.0 mm.

Anomalies in the Branchings of the Stem Bronchi.—Aeby,[10a] Narath[10b] and Flint[6] investigated the early branchings of the stem bronchi: The branchings are comparatively few in number, they occupy definite

11. Cited by Schneider.[2]

12. Yampolsky, J., and Fowler, C. D.: Arch. Pediat. **55**:293, 1938. Jamuni, A., and Ellis, A. G.: Am. J. M. Sc. **196**:824, 1938.

13. Hurwitz, S., and Stephens, H. B.: Am. J. M. Sc. **193**:83, 1937.

14. Kollmann, J.: Handatlas der Entwicklungsgeschichte des Menschen, Jena, Gustav Fischer, 1907, vols. 1 and 2.

15. Grosser, C., in Keibel, F., and Mall, F. P.: Manual of Human Embryology, Philadelphia, J. B. Lippincott Company, 1912, vol. 2, p. 446.

positions and they appear in a predictable sequence. The branches connecting with a stem bronchus are called main bronchi and fall into two groups: ventral bronchi, which are larger and earlier in appearing, and dorsal bronchi, playing a somewhat secondary role. For the more conspicuous malformations of the lung, only the first three or four main bronchi are of interest; these are all of the ventral type.

The first main bronchus becomes recognizable in the right stem bronchus as a bud with ventral direction. The corresponding bronchial projection in the left lung arises only after a definite interval of time, approximately when the right stem bronchus develops its second sprout. The latter is located close to the tracheal bifurcation and subsequently drains the apex of the right lung. Furthermore, it is the only bronchus to course over the (right) pulmonary artery and therefore has been named the eparterial bronchus. Most authors have considered this triple division of the right bronchial system as an arrangement fundamentally different from that found on the left side, where at this stage only two segments of the bronchial system can be distinguished. In order to preserve the analogy between the right and the left lung, Narath suggested that the right apical bronchus should be described as a dorsal main bronchus which has dissociated itself from the first ventral main bronchus and has assumed an earlier and more independent growth. Regardless of any evaluation of the status of this bronchus, the division of the right bronchus into three and of the left bronchus into two projections at this stage, which is usually attained in a human embryo of 7 mm. length, remains permanently reflected in the lobe formation of the lungs. The mesenchymal lung anlagen, surrounding the epithelial structures in the right and left cavities of the chest like mantles, undergo at this moment of the development indentations paralleling the five divisions of the epithelial organ. Both lower lobes form around, and as dependencies of, the stem bronchi. The middle lobe of the right lung arises from the first ventral main bronchus of the right stem bronchus, and the upper lobe of the left lung from the corresponding main bronchus of the left stem bronchus. The right upper lobe finally develops around the eparterial bronchus.

Other main bronchi develop later from the stem bronchi, on both the right and the left side, a few of them taking a dorsal direction. They remain, however, without influence on the configuration of the lung surface. Neither is the appearance of secondary bronchi, i. e., bronchi branching off from the main bronchi, reflected in any way in the external form of the lungs. It is therefore understandable that only occasional observations have been recorded on variations or anomalies in the subsequent arborization of the bronchial tree, because the detection of anomalies in the later developing bronchi would require injection and painstaking dissections.

This interdependence between lobe formation and the earliest branching of the stem bronchus is well illustrated by a typical anomaly. If the eparterial bronchus fails to develop, its place is usually taken by a branch developing at a later time from the first ventral main bronchus of the right lung. In this case the upper and middle lobes may be fused. Conversely, an apical bronchus in the left lung directly connecting with the stem bronchus, repeating the eparterial bronchus formation characteristic for the right lung, is frequently associated with a trilobed left lung (Schneider [2]).

Cystic Disease and So-Called Congenital Bronchiectasis.—It is becoming increasingly questionable whether this deformity is always teratogenic or even congenital. Because of the uncertainty of the genesis of the anomaly in many instances, a purely descriptive terminology seems to be preferable. Such classification may indicate the size of the cystic spaces, their number and the extent of their distribution. The question of communication with the bronchial passages is also important, especially clinically, since cysts isolated from the bronchi are less prone to become infected. With these criteria in mind, the pathologic change encountered may be described as honeycomb lung if the cysts are small and multiple and as air cysts or pneumatoceles if the cysts are large and few. In cases of either type, connections with the bronchi are often not demonstrable.

With few exceptions, the spaces in cystic lungs have been described by the various investigators as dilated air paths, which explains the commonly used but not always correct alternative term of congenital bronchiectasis. Quite often the cyst walls have a very simple histologic structure, lacking entirely the various tissue elements found in the walls of the bronchi. There is, however, no inconsistency in the contention that such cysts are bronchiectases, for it must be remembered that bronchi of less than 1 mm. diameter have normally a much simplified structure, and the most peripheral branches are merely tubes without epithelium, muscle fibers, glands or cartilage.

Differentiation between cystic lung and ordinary bronchiectatic disease is often a difficult task. If we define the latter condition as resulting from mechanical distortion of originally normal bronchi by pathologic processes within the bronchi or in their vicinity, a decision is comparatively easy only in cases in which pulmonary disease processes are in evidence and obviously constitute the cause of the bronchial dilatation. In other cases it will be difficult to exclude the possibility that inflammatory processes are superimposed on a primary malformation. Differentiation between primary and secondary infection is not the only difficulty. Even in the absence of any inflammatory changes the problem of pathogenesis is by no means reduced to one possibility. Most prob-

ably, cystic malformation of the air paths can be produced by a variety of factors quite dissimilar in nature, yet leading to identical end stages.

A priori, we may assume that dilatation takes place if the number of the lining cells in a growing air path increases at a more or less normal rate while the organizing factors normally effecting a budding of side branches or the elevation of folds are in abeyance. It is evident that the cell division under such circumstances must lead to an excessive expansion of the lumen of the affected bronchus.

In another manner, the dilatation may be brought about vicariously, as a sort of horror vacui expansion, if adjacent pulmonary tissue did not develop or, owing to inadequate aeration, did not assume its proper volume. A third possible explanation could be that it resulted from a segmental narrowing with stagnation of secretion or of air (the inspiratory force is greater than the expiratory one).

The enumerated mechanisms could be due to either congenital factors, i. e., vitia primae formationis, or to extrinsic factors like transitory inflammation, disturbance of growth from nutritional deficiency or any other hypothetic noxae. The wide theoretic choice of possible causes for the cystic dilatation of bronchi explains the difficulty in determining the genesis in the individual case, even if the picture is not complicated by secondary inflammation.

Naturally, the factors causing noncicatricial dilatation must be more effective in growing lungs and especially in lungs where the branching out of the bronchial tree has not been completed. This seemingly leads to the conclusion that a cystic condition of the lungs is, after all, a congenital, although not necessarily a primary, malformation. However, recent anatomic studies indicate that the lungs are by no means fully differentiated at birth as Kölliker and His had assumed. The considerable volume increase of the organ during the postnatal growth period is not entirely accounted for by the expansion of the ramifications of the bronchial tree existing at birth. Newer investigations by Broman [16] apparently proved that the branching of the bronchial tree continues in the human lung after birth. Willson,[17] Struckow [18] and Heiss [5] have corroborated Broman's findings. According to Struckow, new ducts and alveoli are formed as late as seven years after birth. Thereafter, the increase in the size of the lungs is accounted for solely by greater size of the air spaces. It seems that the existence of postnatal differentiation in the lung is of considerable importance for the understanding of the genesis of cystic disease. Miller,[19] as far as we are aware, is the only

16. Broman, I.: Verhandl. d. anat. Gesellsch. **32**:83, 1923.
17. Willson, H. G.: Am. J. Anat. **41**:97, 1928.
18. Struckow, A. I.: Ztschr. f. Anat. u. Entwcklngsgesch. **98**:348, 1932.
19. Miller, J. A.: J. Thoracic Surg. **3**:246, 1933.

author who has referred to these rather recent anatomic contributions in the discussion of the etiology of this disease. He pointed out that prenatal and postnatal inflammations, although not producing lasting tissue changes, may interfere with the normal differentiation of pulmonary parenchyma, with the effect that the air paths are reduced in number and increased in size.

Even though the factor of postnatal development has not been emphasized, we find in several recent clinical studies a more critical attitude toward the congenital theory (Romhanyi and Maccone;[20] Pierce and Dirkse;[21] Dey;[22] Nolte[23]). For instance, Dey distinguished three types of bronchiectasis: (1) genuine congenital malformation; (2) bronchiectasis due to tissue predisposition, i. e., weakness of the elastic or of the muscular layer of the bronchus; (3) bronchiectasis due to severe pulmonary inflammation. Evidently the second group provides a convenient middle ground for numerous cases in which a teratologic explanation is not supported by circumstantial evidence, and cicatricial or obstructive changes as in the third group are not prominent.

The histologic observations in cystic disease have not been uniform, and this fact has contributed to the confusion in classification and the difficulties in arriving at a comprehensive understanding of the pathogenesis. The cyst lining may vary from a flattened-out cell layer of indefinite derivation to cuboid and tall columnar epithelial cells unmistakably identical with those found in the bronchial mucosa. The stroma likewise exhibits variability. Muscular elements of the smooth type were, for instance, conspicuous in cases described by Dejerine and Sottas[24a] and by Davidsohn;[24b] in others the connective tissue was hyperplastic, or there was an unusual amount of cartilage. In some cases the authors were impressed by the increased volume of the cystic lung and were led to assume that there was neoplastic hyperplasia of the bronchus (bronchoadenoma).

The wide variety of findings and the difficulty of their interpretation are probably best illustrated by a short synopsis of the various theories proposed for the explanation of cystic disease of the lung and proclaimed as either generally applicable or valid only for specific cases:

1. Primary agenesis of finer ducts and alveoli (Wollmann;[25a] Hückel;[25b] Grawitz;[25c] DeLange[26]). The cysts are passive dilatations of originally normal air ducts.

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20. Romhanyi, G., and Maccone, V.: Frankfurt. Ztschr. f. Path. **50**:442, 1937.
21. Pierce, C. B., and Dirkse, P. R.: Radiology **28**:651, 1937.
22. Dey, H.: Frankfurt. Ztschr. f. Path. **53**:347, 1939.
23. Nolte, F. A.: Ergebn. d. inn. Med. u. Kinderh. **52**:236, 1937.
24. (*a*) Dejerine, J., and Sottas, J.: Rev. de méd., Paris **13**:721, 1893. (*b*) Davidsohn, C.: Berl. klin. Wchnschr. **44**:33, 1907.

(Footnotes continued on next page)

2. Neoplastic hyperplasia of the bronchial tree; "cystic broncho-adenoma" (Stoerk;[27] Wermbter;[28a] Alth[28b]).

3. Hydropic dilatation of congenitally malformed lymph spaces (Virchow; Klebs. See De Lange[26]).

4. Congenital deficiency of elastic tissue (von Hansemann; Dey[22]).

5. Secondary dilatation of air-containing ducts due to persistence of congenital atelectasis (Heller;[29a] Sauerbruch[29b]).

6. Fetal bronchopneumonia, mostly syphilitic, leading to stasis in the bronchi (Balzer[30a]) or to arrested alveolar development (Sandoz[30b]).

7. Lobular pneumonia and pneumonitis in childhood, disturbing the differentiation of the pulmonary parenchyma (Miller[19]).

8. Uncoordinated excessive growth of the pulmonary stroma, interfering with the proliferation of the parenchyma, a hamartomatous neoplasia in the sense of Albrecht (Kimla[31]).

9. Stenoses in short segments of the air ducts developing in the prenatal or postnatal period (Eloesser[32]).

The following conclusions may be permitted concerning the status of cystic disease of the lung among pulmonary malformations:

Cystic lungs are of comparatively frequent occurrence—so frequent indeed as to cast doubt on the probability that they are always primary malformations.

A convenient classification distinguishes between the multiple small cysts, i. e., honeycomb lung, and the large cysts, few in number, i. e., pneumatoceles.

In the vast majority of cases the cysts are dilated branches of the bronchial tree.

Difficulty might be encountered in differentiating lung cysts from bronchiectases produced by scarring or obstruction.

Lung cysts might be produced by unknown teratogenetic factors and by a variety of prenatally and postnatally acquired disease processes. The malformation is therefore not always a primary one.

In rare instances cystic pulmonary tissue is neoplastic.

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25. (*a*) Wollmann: Inaug. Dissert., Freiberg, 1891; cited by Hückel. (*b*) Hückel, R.: Frankfurt. Ztschr. f. Path. **35**:320, 1927. (*c*) Grawitz, P.: Virchows Arch. f. path. Anat. **82**:217, 1880.

26. De Lange, C.: Acta pædiat. **6**:352, 1927.

27. Stoerk, O.: Wien. klin. Wchnschr. **10**:25, 1897.

28. (*a*) Wermbter, F.: Virchows Arch. f. path. Anat. **255**:26, 1925. (*b*) Alth, H.: Frankfurt. Ztschr. f. Path. **30**:463, 1924.

29. (*a*) Heller, A.: Deutsches Arch. f. klin. Med. **36**:189, 1885. (*b*) Sauerbruch, F.: Arch. f. klin. Chir. (Kongressbericht) **148**:721, 1927.

30. (*a*) Balzer, F., and Grandhomme, A.: Rev. mens. d. mal. de l'enf. **4**:485, 1886. (*b*) Sandoz, E.: Beitr. z. path. Anat. u. z. allg. Path. **41**:495, 1907.

31. Kimla, I.: Compt.-rend. XII. cong. internat. de méd. **2** (pt. 3):161, 1897.

32. Eloesser, L.: J. Thoracic Surg. **1**:194, 1931; **1**:270, 373 and 485, 1932.

EXCESSIVE FORMATIONS IN THE DEVELOPMENT OF THE LUNGS

Azygos Lobe.—It has been pointed out that the formation of the lobes is related not only in time but also causally to the development of the first main bronchi, springing from the stem bronchus. Accordingly, in several instances lobe anomalies were found associated with deviations from the normal pattern of the stem bronchus branching. It was also mentioned that for the malformations of large bronchi, and consequently for lobe anomalies, usually no extraneous causes could be detected. The malformations are apparently inherent in the anlage of the lung.

One notable exception to this rule is the so-called azygos lobe. The formation of this seemingly excessive structure is the result of a mechanical interference with the development of the upper lobe. The azygos lobe does not form "spontaneously" but is split off from the mesial aspect of the right upper lobe by the azygos vein if and when this vessel curves in too lateral a direction from the prevertebral region to the anterior mediastinum in order to connect with the superior vena cava. The lateral deviation of the vein is evidently a persistency of a more primitive stage. Inasmuch as the vein is extrapleurally located, its curving through the cavity of the chest necessitates the lifting off of a fold of pleura from the posterolateral thoracic wall. This pleural fold which contains the vessel in its free margin is called the "mesoazygos." The lobe, therefore, is not a supernumerary structure but a part of the upper lobe.

Graham, Singer and Ballon [33a] have reviewed extensively the literature on the accessory lobe of the azygos vein. From their survey we gather that most of the recent contributions are studies based on roentgen examinations. By this method the occurrence of this anomaly has been estimated as between 0.07 and 0.08 per cent. A more recent report by Bacanu [33b] gives 18 azygos lobes diagnosed, 2 of them on the left side, among more than 5,000 roentgen examinations, or in approximately 0.4 per cent.

The azygos lobe may become the seat of pathologic change. Müller [34] found bronchiectasis produced by the pressure of the azygos vein on the bronchus, and Popescu and Secarea [34b] found tuberculosis confined to this lobe.

Supernumerary Lobes.—Most of the supernumerary lobes receive scant attention because no particular pathogenetic principle appears

33. (*a*) Graham, E. A.; Singer, J. J., and Ballon, H. C.: Surgical Diseases of the Chest, Philadelphia, Lea & Febiger, 1935, p. 766. (*b*) Bacanu, C.: Ztschr. f. Tuberk. **80**:355, 1938.

34. (*a*) Müller, H., in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1928, vol. 3, pt. 1, p. 577. (*b*) Popescu, I. M., and Secarea, V.: Cluj. med. **18**:300, 1937.

involved if more than the expected two lobes are found in the left and more than three in the right lung. It is still unknown why ordinarily the monopodial branching of the stem bronchi into main bronchi is followed by a lasting demarcation of the tributary lung mesenchyma only in the case of the first three main bronchi and not in the subsequent ones. Additional fissure formation would mean that the formative principle which regulated the typical division into lobes has remained in effect for a longer time, or that some of the younger main bronchi have developed earlier or more "forcefully."

Among the more typical excess formations can be counted a left apical lobe if its development can be explained, as previously described, by the occurrence of an anomalous eparterial branch of the left stem bronchus. At the base of the lung an excessive lobe is sometimes formed at the mesial aspect. Such a lobe represents a segment of the lower lobe, which normally is drained by a bronchus of larger lumen and of typical course and connection, the infracardiac bronchus. In the right lung this bronchus really has the status of a main bronchus, because it arises directly from the stem bronchus, while in the left lung it is a branch of the second ventral main bronchus. Pozzi has pointed out that in quadrupeds lobe formation around the infracardiac bronchus is quite the rule, at least on the right side. Significantly, in man an abnormal infracardiac lobe is much more frequent on the right than on the left side.

Supernumerary and Accessory Lungs.—Two abnormal formations, each with a separate teratogenesis and a diametrically opposite location, have received—somewhat arbitrarily—the name "supernumerary" or "accessory" lung.

(*a*) Tracheal Lobe: Usually called supernumerary apical or tracheal lung, this rare anomaly is, we believe, more correctly interpreted as a tracheal lobe. The supernumerary formation is supplied by a bronchus arising cephalad to the right apical (eparterial) bronchus, above the bifurcation, i. e., by a bronchus originating from the trachea. In the 2 cases reported in the literature [35] the tissue of the accessory organ resembled aerated normal lung. Not quite as rare as tracheal lobes are certain tracheal diverticula, of which Mackenzie [36a] and Chiari [36b] could collect 6 in their own autopsy material. Apparently these blindly ending bronchus-like projections represent potential tracheal lobes. There is no agreement as to whether the tracheal diverticulum and tracheal lobe should be explained on the basis of a division of the primitive respiratory organ into three stem bronchi or as a supernumerary right apical main bronchus with or without a dependent lobe. Phylogenetic considerations

35. Herxheimer, G.: Centralbl. f. allg. Path. u. path. Anat. **12**:529, 1901. Müller, H.: Virchows Arch. f. path. Anat. **225**:284, 1918.

36. (*a*) Mackenzie, I.: Wien. med. Jahrb. **5**:71, 1881. (*b*) Chiari, H.: Beitr. z. path. Anat. u. z. allg. Path. **5**:329, 1889.

favor the latter theory. A second eparterial main bronchus on the right side is frequent in cetaceans and other mammals. This would make the tracheal lobe an atavistic anomaly. Occasionally in man a double eparterial bronchus is observed supplying a normal right upper lobe. We believe that the bronchus supplying the supernumerary organ is equivalent only to a main bronchus and not to a stem bronchus. Therefore, the tributary lung tissue would represent a lobe and not a lung. In accordance with the law governing the formation of the lobes, the pathogenesis may be explained tentatively along the following lines: an apical or tracheal lung, more correctly a tracheal lobe, develops if a supernumerary eparterial bronchus (probably an atavistically predetermined anomaly) develops sufficiently strong and at a time when the pulmonary mesenchyma is still responding with permanent segmentation (lobe formation) to the epithelial projections.

In spite of the extreme rarity of tracheal lobe formation, von Haberer [37] believed that he could recognize this anomaly clinically. He removed a cystic tumor of considerable size from the upper right interlobar fissure. Because a postoperative roentgen examination showed a bronchus apparently originating above the bifurcation and running toward the site of the removed cyst, he felt justified in assuming that the cyst represented an accessory apical lung.

(*b*) So-Called Lower Accessory Lung: If the tracheal lobe, owing to its location, may be confused with an azygos lobe, the inferior accessory lung must be differentiated from an accessory lobe tributary to an infracardiac bronchus. However, an infracardiac lobe is more frequent on the right, while the lower accessory lung is with few exceptions at the left lung base. We use the term "lower accessory lung" only as a matter of convenience, because here again considerable doubt remains as to whether the designation "lung" is justified. One of the most striking findings, and one that could serve as a diagnostic characteristic differentiating this anomaly from infracardiac lobe, has been the absence of any bronchial communication with the normal lungs. Nevertheless, bronchi unconnected with the bronchial tree, and sometimes of a larger caliber, are an integral part of the supernumerary organ. Microscopically, the tissue is of the pulmonary type, with bronchi and alveoli developed in varying degrees. Similar masses of pulmonary tissue have been found below the diaphragm, i. e., in the abdominal cavity, with or without associated defects in the diaphragm. These so-called abdominal lungs were located more or less in the region of the left kidney, and when all cases are correlated there is little doubt that the so-called abdominal lungs are related to the lower accessory lungs above the diaphragm. Therefore, the abdominal lungs are usually counted among the inferior accessory lungs. Bert and Fischer [8] reviewed 17 cases of

37. von Haberer, H.: Zentralbl. f. Chir. **65**:2134, 1938.

accessory lungs; in only 1 instance was the accessory organ in the right pleural cavity; in 4 it was below the left side of the diaphragm. The size of the organ varied from that of a hazelnut to that of a man's fist, and often the structure was a cystic one. The number of reported cases has now increased to over 30. The more significant findings are listed in the accompanying table.

The problem of the derivation of lower accessory lungs has given rise to three theories. In certain cases the absence of one of the large

Cases of So-Called Lower Accessory Lung

Author *	Side		Diaphragm		Diaphragmatic Hernia
	Right	Left	Above	Below	
Beneke	..	+	..	+	+
Bert and Fischer	..	+	+	..	..
Carter, H., and Osborn, H. A.: J. Obst. & Gynaec. Brit. Emp. **43**: 1194, 1936	..	+	+	..	..
Cockayne and Gladstone	..	+	+	..	+
Freedlander, S. O., and Gebauer, P. W.: J. Thoracic Surg. **8**: 581, 1939	..	+	+	..	..
Kohn	..	+	+	..	+
Duerck	+	..	+	..	..
Gruber	..	+	+	..	+
Hückel	..	+	+	..	..
Humphry	..	+	+	..	..
Kaplan	..	+	+	..	..
Kaup I	..	+	+	..	+
Kaup II	..	+	+	..	+
Lewisohn	..	+	+	..	..
Müller	..	+	+	..	As an inclusion of the lower lobe
Muus	..	+	+	..	..
Pineda	+	..	+	..	..
Quenzel	..	+	+	..	..
Rectorzik	..	+	+	..	..
Robsmann	..	+	..	+	..
Rokitansky	..	+	+	..	..
Ruge	..	+	+	..	..
Sachs	..	+	+	..	..
Scheidegger	..	+	+	..	..
Seitsam	..	+	..	+	..
Simpson	+	..	+	..	..
Springer	..	+	+	..	..
Vogel: case 1 (Dubler)	..	+	..	+	..
Vogel: case 2	..	+	..	+	..
von Meyenburg	..	+	+	..	..
Wechsberg	..	+	+	..	..

* All cases except those of Carter and Osborn, and Freedlander and Gebauer were cited by Scheidegger.[44]

bronchi in the lower lobe of the left lung could be noted, and the accessory lung seemed to be best explained as representing the missing bronchus with its tributary lung tissue. An observation by Hammar [38] is generally considered to support this theory. Hammar accidentally saw sequestration in the bronchial tree at an early developmental stage. He discovered in the serial sections of an embryo of 11.7 mm. length two anomalies in the left lung field. One was an epithelial cyst, not connected with the bronchus, lying in the pulmonary mesenchyma; the other was a similar cyst, lying outside the pulmonary mesenchyma, in the pleural space, and connected by a small pedicle with the uppermost

38. Hammar, J. A.: Beitr. z. path. Anat. u. z. allg. Path. **36**:518, 1904.

recess of the pleural cavity. Wax plate reconstruction showed that the first ventral bronchus (upper lobe bronchus) was undeveloped, and it seemed quite obvious that both cysts originated from the deficient main bronchus by sequestration. Unfortunately, Hammar's findings concern anomalies in the upper lung field, whereas the accessory lungs except for a few doubtful cases are found at the base of the lung. Furthermore, in the majority of cases the bronchial ramifications were seemingly complete.

Somewhat unusual is a case described by Fischer,[39] in which the accessory lung was, so to speak, embedded in the lower lung. It could be recognized by its different consistency and histologic structure. The bronchi, quite prominent in the microscopic picture, did not connect with the bronchi of the lung. The arterial supply to the mass was furnished both by the pulmonary artery and by the aorta, in the latter case by means of a rather large anomalous vessel. The venous drainage was shunted into the pulmonary veins.

The second theory has been developed by Cockayne and Gladstone.[40] It also has sequestration in view but adds a cause for the separation of the anomalous organ from the lower lobe. On the basis of extensive embryologic studies, these authors gained the impression that during certain phases of the development adhesions of lung blastema to the celom mesothelium or to structures covered by this tissue are quite frequent. In the chick embryo they are bilateral and become permanent; in a rabbit embryo they were found between the left lower lobe and the esophagus. They were also seen on the left side in 2 human embryos, and probably in this species are present only in exceptional cases. It remained undecided whether the adherence is a primary one, i. e., represents an area of incomplete separation of lung mesenchyma from the mesenteric tissue, or whether it is produced secondarily by fusion. This theory also explains with some plausibility the coincidence of diaphragmatic defects and the occasional suggestive organic connection of the accessory lung with the stomach, because prior to the formation of the diaphragm the lungs are adjacent to the organs of the upper part of the abdominal cavity.

The third theory of the origin of the lower accessory lung considers the possibility of a very fundamental developmental error, namely, the formation of a second primitive respiratory tract from the digestive tract. Such a developmental anomaly is theoretically conceivable; all that is probably necessary for its occurrence is a downward displacement of cells from the tracheoesophageal ridge with the growing foregut. Most authors favoring this theory of a separate lung anlage have been influenced by the presence of a blood supply for the accessory lung

39. Fischer, H.: Unpublished Thesis, Düsseldorf; cited by Müller,[34a] p. 582.
40. Cockayne, E. A., and Gladstone, R. F.: J. Anat. **52**:64, 1917.

independent of the pulmonary circulation, which indeed seems to be a constant finding. The accessory lung, whether its location is above or below the diaphragm, is usually supplied by arteries which come either from the aorta directly or from the left adrenal vessels. The veins likewise belong to the general circulation, emptying into the azygos or hemiazygos vein. Only in the case reported by Fischer,[39] already mentioned, and in one reported by Lewisohn [41] was the venous drainage directed into the pulmonary vein, but even in these cases the arterial supply, in part at least, was from the aorta.

Beneke [42] was the first to discount the significance of vascular connections for establishing the origin of accessory lungs. He pointed out that a displaced organ may easily obtain a vascular supply from the vicinity if the ectopic location was assumed early in the development. Recent experiments by Hamburger [43] on chick embryos illustrated this biologic principle very clearly. If limb buds are transplanted into a new position, they may develop to a remarkable degree and will then incorporate blood vessels and nerve fascicles attracted from the segmental nerves and blood vessels of the new location. More difficult to dispose of as an argument in favor of the theory of a second primary respiratory tract are the observations by Beneke,[42] Muus, Kaplan and Scheidegger,[44] who found in the pedicle of the accessory lung epithelium-lined ducts connecting the organ, as it appeared, either with the stomach (in some instances the accessory lung was nevertheless located above the diaphragm) or with the esophagus. Of these authors, Beneke and Kaplan were inclined to consider the connection with the digestive tube, which, by the way, has never been found to be complete, as a secondary occurrence, similar to the attachment of blood vessels from the aorta or the adrenal vessels to the accessory lungs. Among the more recent contributions to this subject, Scheidegger's [44] case report is of interest. He gave a detailed description of a pedicle. Running from the cardia of the stomach, but not communicating with its lumen, it penetrated through the diaphragm. From the stomach up to the diaphragm, the connecting tube had the structure of a narrow esophagus, with the difference that the mucosa was of the gastric type. Yet, as emphasized before, the mucosa remained separate from the lining of the stomach. After penetrating the diaphragm, the muscular layers became less coherent, and the mucosa blended with one of the several bronchial tubes which were numerous in the supradiaphragmatic portion of the pedicle. The arterial vessels came from the aorta and the adrenal arteries; there was a single vein, of larger caliber than the arteries, connecting with the hemiazygos vein. Remarkably, the accessory lung

41. Lewisohn, cited by Bert and Fischer.[8]
42. Beneke: Verhandl. d. deutsch. path. Gesellsch. **9**:202, 1905.
43. Hamburger, V.: J. Exper. Zool. **80**:347, 1939.
44. Scheidegger, S.: Frankfurt. Ztschr. f. Path. **49**:362, 1936.

contained air, while the normal lungs were completely atelectatic (the patient was a newborn baby which lived only a few seconds but had made several respiratory efforts during this time). Scheidegger did not enlarge on the question of how air could have got into the alveoli of the accessory lung, but the photomicrographs show convincingly expanded alveoli.

Ectopic Lungs.—While in the cases of inferior accessory lung an origin from a low pulmonary anlage, developing from the esophagus or stomach, is neither generally accepted nor even suggested in the majority of the cases, there are a few isolated observations on hand indicating that such displacement of formative cell material, with its natural consequence of anomalous lung development, has occurred.

Klebs [45] described a right lung represented by a small bronchus-like projection of the lower part of the esophagus ending in a small mass of pulmonary tissue. The left lung was normally developed and in connection with the trachea and larynx. Beneke [42] reported a case in which the primordial respiratory tract at its typical location had produced only an undersized larynx. The lungs, however, took their origin independently, from the cardiac end of the esophagus, with two stem bronchi and a bifurcation. Although the lungs were small, their histologic appearance was quite normal.

Apparently, these malformations are the result of disturbances in the tracheoesophageal separation. Other more common results of developmental errors during this process are the various types of tracheoesophageal fistula, a subject which is not within the scope of this paper.

Summarizing the descriptions of so-called accessory and supernumerary lungs, we find that they concern two different types of congenital anomaly: Extremely rare is a lobe in the right apical region connected by an anomalous tracheal bronchus with the tracheobronchial tree. More commonly, the so-called accessory lung is a mass of pulmonary tissue at the base of a lung, occasionally located even below the diaphragm, lacking bronchial connections with the lungs. In either case the term "lung" is misapplied if the more plausible theories of the origin of the anomaly are credited. If we define as a lung that part of the respiratory tract that contains a stem bronchus in its entirety, both modalities of excessive or abnormally segregated lung tissue can be considered at best as lobes and in the case of "lower accessory lung," only as parts of a lobe.

On the other hand, 2 instances of lung anomalies unmistakably due to a displaced anlage of a stem bronchus or even a displaced bifurcation have been described. For them the term "lung" is appropriate, and we propose to call them ectopic lungs.

45. Klebs, E.: Aerztl. Cor.-bl. f. Böhmen. **2**:111, 1874.

News and Notes

University News, Promotions, Resignations, Appointments, Etc.—Henry F. Vaughan, health commissioner of Detroit, has been appointed professor of public health in the University of Michigan. A new school of public health will, it is hoped, be placed in operation next fall.

A cancer research institute has been established in the Hebrew University of Palestine.

The laboratory of research pathology under the direction of Leo Loeb at Washington University, St. Louis, will be closed June 30, 1941.

A division of chemotherapy has been formed in the National Institute of Health of the United States Public Health Service, under the direction of William H. Sebrell Jr. The new division will be concerned especially with research on sulfanilamide products and on the synthesis of substances with antimalarial properties.

Recent Deaths.—Charles Wardell Stiles, formerly medical zoologist of the United States Department of Agriculture Bureau of Animal Industry, and later of the United States Public Health Service, pioneer investigator of hookworm disease, died on January 24, 73 years old.

Max Askanazy, professor emeritus of pathologic anatomy in the University of Geneva, Switzerland, died Oct. 23, 1940, at the age of 75 years. Askanazy was born in East Prussia and began his academic career at the University of Königsberg, joined the faculty of medicine of Geneva, Switzerland, in 1905 and served there until his retirement in 1939. His special fields of research were diseases of the blood, malignant tumors and diseases of bones. He was an excellent teacher. He founded the International Society for Geographic Pathology. In 1935 he was made an honorary citizen of Geneva.

William Webber Ford, professor emeritus of bacteriology in the Johns Hopkins University, died Feb. 10, 1941, at the age of 70.

William Bulloch, professor of bacteriology in the University of London, died on February 11, in his seventy-third year.

Society News.—The Federation of Societies of Experimental Biology, composed of the American Physiological Society, the American Society of Biological Chemists, the American Society of Pharmacology and Experimental Therapeutics, the American Society for Experimental Pathology and the American Institute of Nutrition will meet in Chicago from April 15 to 19. The Stevens Hotel has been chosen as headquarters, and all scientific sessions and demonstrations will be held there.

The next annual meeting of the American Society of Clinical Pathologists will be held in Cleveland, May 30 and 31 and June 1, 1941. The headquarters will be Hotel Cleveland.

Grants for Sex Research.—Applications to the Committee for Research in Problems of Sex, National Research Council, for financial aid during the fiscal year beginning July 1, in support of work on fundamental problems of sex and reproduction, should be received before April 1. They may be addressed to the chairman, Dr. Robert M. Yerkes, Yale School of Medicine, New Haven, Conn. Although hormonal investigations continue to command the interest and support of the committee, preference, in accordance with current policy, will ordinarily be given to proposals for the investigation of neurologic, psychobiologic and behavioral problems.

Book Reviews

Clinical Pellagra. Seale Harris, M.D., professor emeritus of medicine, University of Alabama, assisted by Seale Harris Jr., M.D., with foreword by E. V. McCollum, Ph.D., Sc.D., LL.D., professor of biochemistry, Johns Hopkins University School of Hygiene and Public Health. Cloth. Pp. 494, with 65 illustrations. Price $7. St. Louis, Mo.: C. V. Mosby Company, 1941.

Pellagra is a disease that has come to interest the entire medical profession. The studies of recent years have indicated that this disease is present throughout the civilized world with a few possible exceptions. The advent of the biochemist in this field of study has assured a sound approach to the basic problems of nutrition in general and pellagra in particular. The reward of a rational treatment in the control of this disease has been particularly heartening.

The author in his preface states his objective as "a factual treatise on pellagra." In general he adheres to this particular aim; in his occasional departures he loses weight by philosophies that are not based on clinical analyses of the available facts or even on sound logic. For example, the attempt to show an analogy between pellagra and diabetes mellitus is scarcely justifiable in the light of the respective underlying deficiencies and pathologic changes incident thereto. The author subscribes to the belief that gastric and hepatic intrinsic factors initiate or contribute to the nicotinic acid deficiency and defends his position vigorously. Unfortunately, the illustrations that are used to demonstrate the comparable lesions of the liver and the spinal cord in pernicious anemia and pellagra (pages 162, 165, 167) are not particularly clear.

The clinical discussions are sound but suffer from repetition. The expedient of the inclusion of published articles (Porter and Higgenbotham on the heart; Sutton and Ashworth on the interrelations between vitamin and hormone deficiencies) is certainly unusual. Interesting as are these chapters and the ones contributed by Ruffin and Smith and by Dodd, there is a distinct loss of the personal element that lends continuity to the remainder of the text. In fact, the entire manner of presentation, including the references, changes. An impression of padding is gained from this source and from the illustrations that repeat those offered by the author himself. Closer editorship would have eliminated such duplication in text and illustrations. Interesting as are isolated case citations from the experience of as able a clinician as the author, the inclusion of such details as "Suicide of Convalescent Pellagrin After Exposure to Sunlight" (pages 136-138) and "My Case of Pellagra, Sprue and Pernicious Anemia" (pages 172-173) in a text of this type is scarcely justified.

Equally inexplicable errors are included, as the spelling of the name of Louis B. Hamman of the Johns Hopkins University as "Hammon" (page 21), Charles Wardell Stiles as "Styles" (page 45), Harvey W. Wiley as "Wyley" (page 387) and C. P. Rhoads as "Rhode's" (page 19). Interestingly, the latter two errors are corrected in the bibliography and Stiles is correctly spelled later in the text (page 401). Furthermore, H. W. Harris is credited with the first demonstration of the hookworm in this country, in 1904 (page 179), whereas this distinction properly belongs to the late Allen J. Smith (1895).

Obviously a crusader, Dr. Harris stands in his strongest light in the concluding chapters on "Prophylaxis" and "Treatment." The necessity for balanced diets rather than inadequate diets fortified by vitamins is stressed. The author lays heavy emphasis on the responsibility of alcoholic indulgence in the perpetuation of pellagra. He castigates the devitaminized foods and welcomes the passing of the yeast fad. He refers repeatedly to the multiplicity of factors involved in any deficiency and strongly urges the wisdom of an educational program that extends beyond the actual food stuffs to the manner of farming. With regard to his own community, he strongly urges the cotton farmer to cultivate a garden patch and to raise "corn, wheat, vegetables, fruits, chickens, hogs, dairy and other food products at home."

Books Received

ELECTROCARDIOGRAPHY IN PRACTICE. Ashton Graybiel, M.D., instructor in medicine, courses for graduates, Harvard Medical School; research associate, Harvard University Fatigue Laboratory; assistant in medicine, Massachusetts General Hospital. Paul D. White, M.D., lecturer in medicine, Harvard Medical School; physician, Massachusetts General Hospital. Cloth. Pp. 319, with 272 illustrations. Price $6. Philadelphia and London: W. B. Saunders Company, 1941.

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